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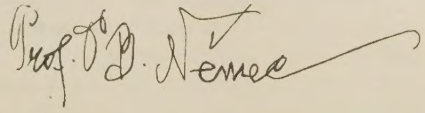
The appearance of this first number of our new journal marks the fulfilment of a desire long cherished by Czechoslovak plant physiologists and workers in other branches of experimental botany. Its aim will be to acquaint the scientific public in our country and abroad primarily with the work of our own scientists, who are desirous of making their contribution to the progress of science. In our journal we warmly welcome the co-operation of our colleagues from all parts of the world in the development of international scientific endeavour.

It is our belief that our journal can also make some contribution to our mutual efforts towards realising the dream of the best minds of all nations—the achievement of well-being, health, peace and happiness.



Praha, January, 1959.

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BIOLOGIA PLANTARUM (PRAHA)

1 (1) : 3—8, 1959

Catalase Activity in Thermal Blue-green Algae in Relation to Temperature

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Received April 24, 1957

Souhrn

Autor sledoval aktivitu katalasy termálních sinic v závislosti na teplotě. Jako materiálu bylo použito homogenátu z termálních sinic r. *Oscillatoria* (teplotní optimum 38° C). Manometrická metoda podle APPLEMANA (1951) byla poněkud modifikována, ale princip (stanovení uvolněného kyslíku v prvních fázích reakce) zůstal zachován. Na rozdíl od běžných manometrických metod se během stanovení nezachovává konstantní objem (V_0) a registruje se čas potřebný ke změně tlaku o určitý interval. Výsledky se přepočítávají na V_0 graficky.

Nebyly zjištěny žádné zvláštní vlastnosti katalasy termálních sinic, pokud jde o závislost její aktivity na teplotě, což potvrzuje předpoklad M. B. ALLENOVÉ (1950), že neexistují zvláštní bílkoviny resistentní k vysokým teplotám. Reakční rychlost se v závislosti na teplotě zvyšuje až do 55° C. Při teplotě 60° C je enzym z termálních sinic prakticky kvantitativně denaturován, stejně jako katalasa z mesofilních mořských řas nebo z býčích jater.

Summary

The author has studied the relation of the catalase activity in thermal blue-green algae to temperature. Experimental data were taken from the first phase of the reaction, so that oxidation of the enzyme by the substrate was eliminated. The relation of the activity of a homogenate of thermal blue-green algae to temperature gives a curve similar to that obtained by SIZER (1944) for pure enzyme preparations isolated from beef livers. The resistance of the enzyme to high temperatures is the same in thermal blue-green algae as in mesopholic seaweeds. The catalase of thermal blue-green algae does not exhibit any special characteristics differing from the catalase of mesophiles.

Introduction

The thermophily of some organisms still remains incompletely explained, although we know by now for certain that their metabolism is essentially the same as that of mesophiles, that no natural division with regard to temperature exists between thermophiles and mesophiles and that thermophily is a characteristic which organisms of the same species can gain and lose (*Sporovibrio desulfuricans*).

On the basis of her experiments ALLEN (1950) has come to the conclusion that the nature of thermophily is not to be sought in the specific resistance of plasmatic proteins to high temperatures. In her opinion the more intense metabolism at high temperatures is responsible for the higher resistance of thermophilic organisms, the synthesis of their enzymes and other plasmatic components taking place more rapidly than their denaturation. This assumption has been based on the direct relationship between consumption of nutrients from the environment and rising temperature. With insufficient nutrients thermophilic species or strains *(Bacteria)* perish at high temperatures as quickly as mesophilic species.

Blue-green algae are characterised by the wide temperature limits within which they can exist and the thermophily of many species can be regarded rather as facultative than strict. In connection with the study of the temperature dependence of photosynthesis and respiration in blue-green algae the author also observed the dependence of catalase activity on temperature.

Materials and methods

Thermal blue-green algae of the genus *Oscillatoria* (with optimum temperature for growth, photosynthesis and respiration at temperatures close to 35° C). Both intact algae filaments and homogenate were used. A pure 14-days-old culture of the species chosen cultivated in a small Roux flask (20 ml. medium) was homogenised for 3 minutes in an all-glass homogeniser after POTTER and ELVEHJEM (1936) with 10 ml. phosphate buffer pH = 7 (Na_2HPO_4 M/15 + KH_2PO_4 M/15). In each case 2·7 ml. of this suspension, corresponding to 0·25 mg. of total nitrogen, was pipetted into a manometric flask. By using cultures from eight cultivation dishes enough material was obtained for all tests. By preliminary experiments it was established that the catalase activity of the homogenate was not changed even after 24 hours if kept at 0° C in the dark. This period sufficed for the determination of enzyme activity at all temperatures chosen with a sufficient number of repetitions.

In determining the tempterature coefficient of enzymatic splitting the concentration of the substrate must be chosen with a view to the concentration of the enzyme so that the time dependence of the rate of reaction should be suited to the kinetic equation for "zero-order" reactions which are characteristic for heterogenous catalysis (FRUTON and SIMMONDS 1954). For this reason in the preliminary experiments hydrogen peroxide was added to the suspension of homogenised algae to give resultant concentrations of 0·033 M, 0·1 M and 0·3 M. Preliminary determinations were carried out at a temperature of 20° C. The amount of oxygen evolved in relation to time agreed in the time interval used with the kinetic equation of "zero-order" reactions

$$-dC/dt = kE$$

(where E is the amount of enzyme present) only with a resultant concentration of the substrate of 0·1 M. In this and all other experiments the material was incubated at the given temperature for 10 minutes. The concentration 0·3 M already caused a marked denaturation of the enzyme, while at the concentration of 0·033 M H_2O_2 the reaction took place clearly in accordance with the kinetic equation for a monomolecular reaction

$$-dC/dt = kC$$

In view of the strongly oxidising properties of the substrate it is not possible in the last case to decide to what extent the decrease in reaction rate in the course of time in the last phase is related to the concentration of the substrate or to the denaturation of the enzyme. Therefore, in further experiments, a resultant concentration of 0·1 M and a somewhat modified method after APPLEMAN (1951) were used which made it possible to determine rate constant during the first thirty seconds following the start of the reaction. Manometric determination forms the basis of this method, but the time corresponding to the change in pressure in a given interval is recorded.

With the experiments described this interval was 10 mm. of manometric liquid. The rate of reaction would make it difficult to maintain the volume of gas V_g at a constant level. In order to avoid this difficulty the values obtained were calculated according to the mathematically derived and experimentally confirmed relation

$$h = 2h_1 + \frac{P \cdot V_m}{V_g},$$

where $2h_1$ is the change in pressure in mm. of manometric liquid when the volume of gas V_g is not maintained at a constant level (h_1 is the change in level in one arm of the manometer) and V_m is the volume of gas formed in the capillary above the liquid in the closed arm of the manometer. The remaining symbols are common in manometry.

For the purpose followed Warburg's respirometer of usual type with a vessel lacking the central cup and with one side arm was used.

The above relation is only approximate, as follows from its derivation:

$$pV = (P + h) \cdot V_g \quad (1)$$

$$pV = (P + 2h_1) \cdot (V_g + V_m) \quad (2)$$

By comparison of the right sides of these equations the following expression is obtained

$$h = 2h_1 \frac{V_g + V_m}{V_g} + \frac{P \cdot V_m}{V_g}.$$

Since

$$V_g + V_m \cong V_g,$$

then

$$\frac{V_g + V_m}{V_g} \cong 1$$

and therefore this expression can be ignored in the formula. Within the pressure range of the Warburg manometer it is possible in most cases to ignore the error (less than 3%) so produced. For example, if $V_g = 16{,}850$ l., $\mu h_1 = 300$ mm. and $V_m = 800\ \mu$l. (the volume of gas corresponding to 1 mm. of manometric liquid must be known; in our case it is 2·67 μl.) Calculating h according to the original formula the resultant value is 1105 mm., when using the simplified formula h is 1075 mm.

Within the pressure range of the Warburg manometer the relation between h and h_1 is also practically linear, which can be usefully employed in the graphic evaluation of the results.

Time intervals corresponding to a change in pressure of 10 mm. of manometric liquid were registered photographically. A spring machine was used in which a constant speed was ensured

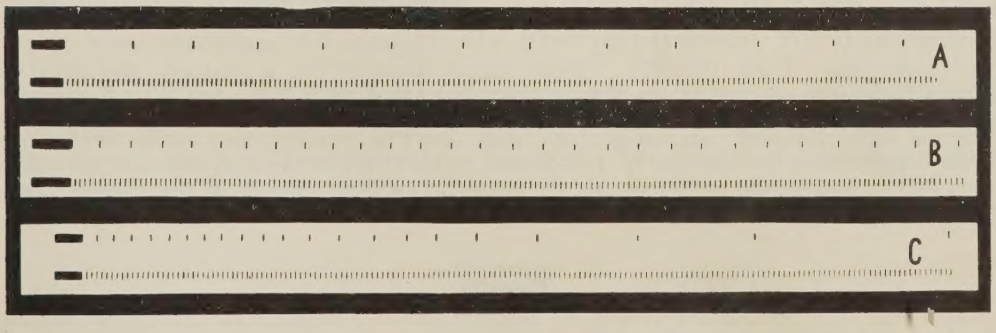

Fig. 1. Photograph of original time records from which the rate of decomposition of hydrogen peroxide by the catalase was worked out.
A) 0° C, B) 30° C, C) 50° C.

by a centrifugal regulator. To the machine there was attached a drum with grooves for the insertion of polarographic paper and with a signalling apparatus to announce the beginning and the end of the record paper. The speed of rotation was one turn in two and a half minutes (r = = 8 cm.). Opposite the drum there was a slit with two signal electric bulbs one above the other. The contacts of metronome were connected to the circuit of one bulb recording the interval of one second. The circuit of the other bulb was connected by the pressing of a switch at the moment

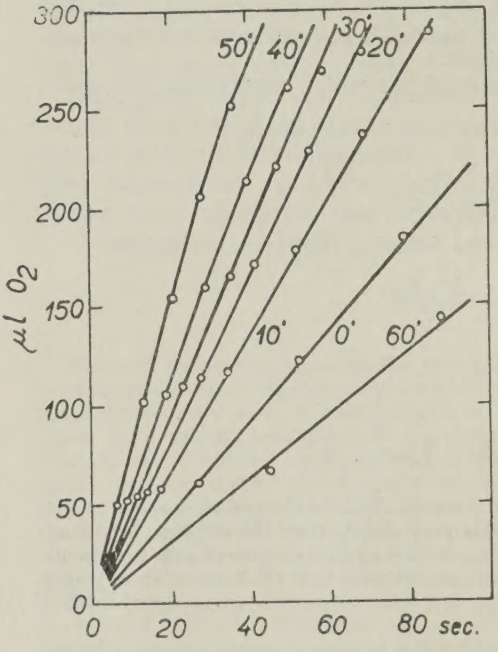

Fig. 2. Rate of liberation of oxygen from 0·1 M solution of hydrogen peroxide at various temperatures. The reaction was catalysed by a homogenate of the alga *Oscillatoria* at pH = 7.

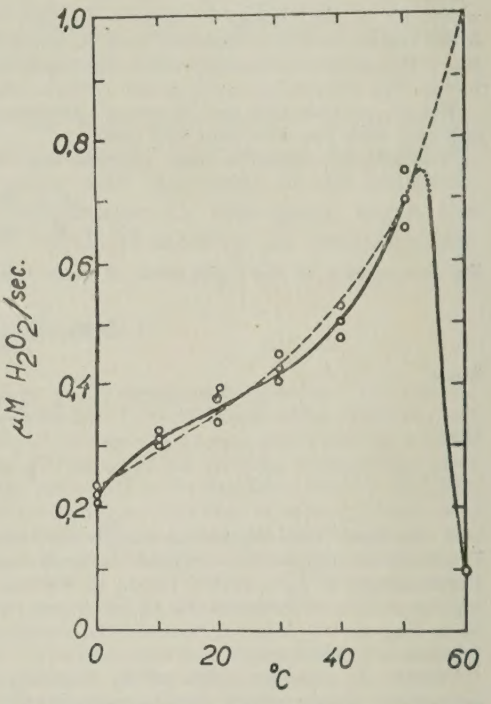

Fig. 3. Relation of the catalytic activity of the *Oscillatoria* homogenate to temperature. The measure of activity of the homogenate is the original rate of decomposition of hydrogen peroxide expressed in micromols of H_2O_2 distributed in seconds.

when a change in pressure of 10 mm. of manometric liquid took place. In this way, after developing the records, two rows of signs were obtained, of which one was a measure of the time in seconds and the other indicated—according to this scale—the time necessary for a change in pressure of the degree chosen to occur (fig. 1). It is possible to read the pressure with sufficient accuracy even during shaking (100 oscillations to the minute). It is best to read it at the moment when the moving meniscus first reaches each fifth graduation of the scale.

Results

The course of the decomposition of hydrogen peroxide in relation to time is given in fig. 2. The relation of the rate constant determined (M H_2O_2/sec.) to temperature is illustrated in fig. 3. Deviations from the theoretically pre-

sumed exponential curve cannot be fully explained since the work was not carried out with the pure enzyme but with a suspension of homogenised material. Therefore, not even the logarithmic expression of the constant in relation to 1/T gives a straight line (fig. 4).

Activation energies for individual temperature intervals were calculated from Arrhenius's equation $= 2{\cdot}303 \cdot R \frac{\log k_2 - \log k_1}{1/T_1 - 1/T_2}$ (where R is the gas constant expressed as 1.987 cal./mol.). They are set out in the following table:

0 to 50° C	4100 cal.
0 to 10° C	5500 cal.
10 to 40° C	2900 cal.
40 to 50° C	6400 cal.
50 to 60° C	59,000 cal.

Experiments with whole, non-homogenised material gave similar results. For obvious reasons their dispersion was, however, rather greater.

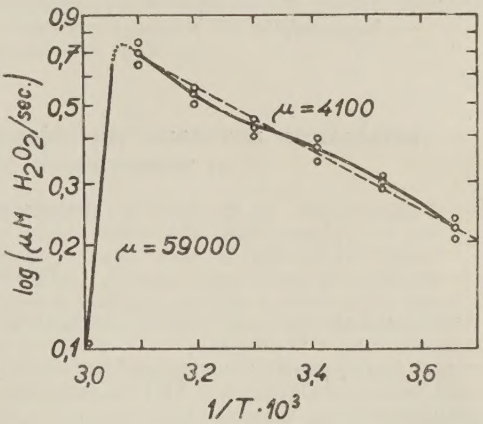

Fig. 4. Relation of the logarithm of the rate constant of decomposition of hydrogen peroxide (catalysed by homogenate of the alga *Oscillatoria*) to the inversion value of absolute temperature at which the reaction took place.

The results obtained do not show any essential differences from these of Sizer (1944), using catalase isolated from beef liver, or from those of the Japanese author Takagi (1953) who worked with five species of seaweeds: *Ulva pertusa*, *Enteromorpha linza* var. *crispata*, *Scytosiphon lomentaria*, *Polysiphonia venticulosa* and *Porphyra pseudolinearis*. As far as the resistance of the catalase to high temperatures is concerned, the results are also numerically in complete agreement both for marine mesophiles and for thermal blue-green algae. Bonnichsen and co-workers (1947) obtained, on the basis of their experiments with very pure preparation, a considerably lower value of activation energy (1700 cal.) and they stated that higher values were obtained only when working with imperfectly pure preparations. For denaturation Sizer has quoted a lower activation energy (51,000 cal.), but this can be explained by differences in method (different incubation period, etc.). It seems, then, that the catalase of thermal blue-green algae does not exhibit any special characteristics as regards the relation of its activity to temperature and resistance to high temperatures.

References

Allen, M. B.: The dynamic nature of thermophily. — J. Gen. Physiol. **33** : 205—210, 1950.

Appleman, D.: Manometric determination of catalase activity. Apparatus and method. — Anal. Chem. **23** (11) : 1627—1631, 1951.

Bonnichsen, R. K., Chance, B. and Theorell, H.: Catalase activity. Acta chem. Scand. 1 : 685—709, 1947.

Fruton, J. S., Simmonds, S.: General Biochemistry. (pp. 235—260). New York, 1954.
Potter, V. R., Elvehjem, C. A.: (cit. after Kleinzeller, A., Málek, J., Vrba, R.: Manometrické methody a jejich použití v biologii a biochemii. Praha, 1954): Manometric methods and their application in biology and biochemistry. — J. biol. Chem. **114** : 495—504, 1936.
Sizer, I. W.: Temperature activation and inactivation of the crystalline catalase-hydrogen peroxide system. — J. biol. Chem. **154** : 461—473, 1944.
Takagi, M.: On the stability against heat of the catalase in marine algae. — Bull. Jap. Soc. Sci. Fish. **19** (8) : 886—888, 1953.

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Активность каталазы термофильных синезеленых водорослей в зависимости от температуры

ШТЕПАН КУБИН

Резюме

Автор исследовал активность каталазы термофильных синезеленых водорослей в зависимости от температуры. В качестве материала применялся гомогенат из термофильных синезеленых водорослей *Oscillatoria* (оптимум температуры 38° С). Манометрический метод Appleman-a (1951) был несколько модифицирован, но его принцип (определение освобождающегося кислорода в первых фазах реакции) оставался неизменным. В отличие от обычных манометрических методов, в течение определений не сохраняется постоянный объем (V_o) и регистрируется время, необходимое для изменения давления на определенную величину. Результаты перечисляются на V_o графически.

Зависимости активности каталазы термофильных синезеленых водорослей от температуры не были отмечены. Это подтверждает предположение M. B. Allen-a (1950), что не существуют особые белки, устойчивые к высоким температурам. Скорость протекания реакции повышается пропорционально повышению температуры вплоть до 55° С. При 60° С энзим из термофильных синезеленных водорослей практически полностью денатурируется, как и каталаза из мезофильных морских водорослей или из бычьей печени.

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Application of Humus Substances to Overground Organs of Plants

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Souhrn

Někteří autoři se domnívají, že humusové látky nepůsobí na rostlinu přímo, nýbrž zvyšují pouze rozvoj kořenové a půdní mikroflory, jejíž působení je pak vlastní příčinou lepšího růstu rostlin. Abychom mohli studovat příznivý vliv humusových látek na růst a metabolismus rostliny, vyloučili jsme jejich působení na půdní a kořenovou mikroflóru tím, že jsme je podávali rostlině postřikem na list. Současně nám šlo o to, zjistit možnost využití příznivého působení humusových látek při postřiku rostlin v praxi.

Na základě předběžných pokusů byla pro další práci vybrána rostlina *Begonia semperflorens* LINK et OTTO, která citlivě reagovala na humusové látky. K postřiku bylo použito dialysovaného extraktu zahradní půdy, obsahující humát sodný s příměsí frakce fulvokyselin v celkové koncentraci 300 mg/l. Jednotlivé dávky, aplikované dvakrát týdně v době hlavní růstové fáze, byly 2 ml.

Bylo zjištěno, že humusové látky aplikované na list působí prodlužování rostlinných orgánů i zvětšení jejich svěží váhy a sušiny. Je zvýšen i obsah chlorofylu a množství vydýchaného kysličníku uhličitého. Konečný efekt závisí na době, kdy byl postřik proveden, i na použité koncentraci. Lze mít za to, že tyto účinky jsou výsledkem bezprostředního působení humusových látek a nikoliv působení stimulované půdní či kořenové mikroflory nebo zlepšených fysikálních, resp. fysikálně chemických vlastností půdního prostředí.

Summary

The authors studied the effect of the application of humus substances to the leaves of *Begonia semperflorens* LINK et OTTO. For spraying they used a dialysed extract of garden soil containing sodium humate with admixture of fulvic acid in total concentration of 300 mg/l. Doses of 2 ml. were applied twice a week during the period of the main growth phase.

It was found that humus substances applied to the leaves cause a lengthening of plant organs and increase of their fresh and dry weights. The chlorophyll content and the amount of expired carbon dioxide are increased. The final result depends on the time when spraying was carried out and on the concentration used. It can be assumed that these effects are the result of direct influence by the humus substances and are not due to the action of stimulated soil or root microflora nor to improvement of the physicochemical character of the soil environment.

Introduction

The favourable influence of humus and humus substances on the growth and development of plants has been demonstrated in the past by many authors and is today more or less generally recognised (extensive literature on this subject was collected by PRÁT 1955). However, the way this influence is exerted, by what paths the humus substances impinge on the flow of metabolic processes or biochemical reactions taking place in the plant organism remained undecided. The fact that humus substances give rise to striking changes in such fundamental vital processes as respiration, glycide conversions, reception of mineral ions, cell division or growth, led to the acceptance as more or less self-evident, of the supposition that these substances enter into organs and cells and there directly influence the metabolic processes. Nevertheless, some authors hold the view (e. g. CHIZHEVSKY and DIKUSAR 1955) that humus substances do not act in this direct manner on the plant, but that they merely stimulate the development of root and soil microflora, the influence of which is then the actual cause of improved plant growth, or that they simply influence the physical and physicochemical conditions obtaining in the soil. It is indisputable that humus substances act both on the soil microflora and on some important qualities of the soil. It is therefore necessary, if we wish to study the direct influence of these substances on plants, to eliminate both these factors of the external environment. This requirement can be partly met by using sterile water or sand cultures. In our work we have chosen another method of eliminating the influence of soil environment and its microflora, that is the application of humus substances to the leaves by spraying. At the same time we wished to determine whether it would be possible to employ the stimulatory qualities of humus substances by spraying plants, as this could be of considerable significance in market gardening and in agriculture.

Material and Methods

We carried out many preliminary experiments, which showed that it is necessary above all to choose suitable experimental material, ascertain the optimal concentration of the preparation to be used and the most favourable time for application with regard to the growth and development of the plant. As a result of these experiments we chose for further work the plant *Begonia semperflorens* LINK et OTTO, which reacted sensitively to humus substances. Results obtained in experiments with this plant are the subject of this paper.

Begonia semperflorens was grown from seed in a glass house to the stage of two large assimilation leaves. The seedlings were then transplanted to flower pots, one set being 11 cm. in diameter with a content of 800 g. of garden soil and the other 9 cm. in diameter with a content of 400 g. of garden soil. From a larger number of these transplanted seedlings 25 pairs corresponding in appearance and growth were selected from those in the large flower pots and 15 pairs from the small pots. In both groups one half was sprayed with humus substances and the other half, serving as control, with distilled water. The plants were kept as before in the glass house. A hand sprayer was used, which formed misty drops. In this way the solution was distributed evenly on the leaves, stems and flowers to form a fine, even coating. Care was taken that the solution should not drip on to the soil in the flower pots. The plants were sprayed in this way twice a week during June and July, i. e. during the period of greatest growth up to the flowering and renewed slowing of growth.

The dose for one spraying of one plant was about 2 ml. of solution of humus substances, concentration 300 mg./l. The solution was prepared as follows: dried garden soil was extracted cold with 96% ethanol in order to separate the alcoholic fraction. After drying it was decalcified with 1% HCl and following thorough rinsing with distilled water extracted with 0·3 N NaOH. The solution was then filtered, concentrated by freezing out and dialysed for 70 hours. Finally the concentrate was diluted to 300 mg. of humus substance in one litre of solution, the final pH of which was 6·5. The composition corresponded to sodium humate with admixture of fulvic acid and other substances of this fraction. For the period during which we used it the solution was kept in a refrigerator at a temperature of —1 to —5° C.

The differences between the experimental plants and the controls were evaluated by measuring the lengths of stems and roots, determining fresh and dry weights of overground parts and roots and finally by measuring the length and breadth of the blades of leaves at each level. The values obtained were averaged and the differences in relation to the controls ascertained. The chlorophyll content was established by the colorimetric method according to Godnev (1952) and the amount of expired carbon dioxide was determined by absorption in barium hydroxide.

Results

The experiments showed that spraying of *Begonia semperflorens* with a solution of humus substances affects growth favourably. This was apparent from the length of stems and roots and the fresh and dry weights of these organs. The values measured and the differences as compared with the control are given in table 1 and photographically recorded on figs. 1 and 2. Humus substances affect both the length and breadth of leaf blades. This is graphically illustrated by fig. 4. Spraying with humus substances also influenced the chlorophyll content and the production of carbon dioxide by leaves in the dark as can be clearly seen from table 2. Overdosing with humus substances through spraying has an inhibitory effect. Growth is slowed up, leaves are wrinkled, branching of the stems occurs. The general appearance of such a plant is shown in fig. 3.

Discussion

The results of experiments with *Begonia semperflorens* show that humus substances affect the whole plant organism even when they are applied to the leaves. At the same time the results of this action do not in general differ from the effects caused by humus substances when present in the soil. There was a considerable increase in the fresh and dry weights of the overground parts and roots and this increase was percentually greater in the case of plants in the small flower pots than in those in the large pots. The dry weights of stems and roots of plants in the large pots remained on the whole within the limits of variability of biological material, but showed significant increases in the case of plants in the small pots (table 1). It is necessary, however, to point out that changes in the length and weight of the root system are undoubtedly distorted to a certain extent by the procedure of washing out traces of earth. Since the root system is very delicate even our great care in preparation could not ensure against the possibility of causing changes in weight. For this reason we regard the changes in the root system recorded in table 1 as preliminary results which will have to be verified in water culture. Both

Table 1. Dimensions and weights of organs of *Begonia semperflorens* the overground parts of which were sprayed with solution of humus substances of total concentration 300 mg./l. Control plants were sprayed with water

		Control plants	Plants sprayed with humus solution	Difference in % of control	Significance t
Plants grown in large pots (25 plants)					
Stems	Height in cm.	27·1 ± 3 . 1·22	33·9 ± 3 . 1·66	25·1**	3·30
	Fresh weight in g.	29·8 ± 3 . 1·21	37·3 ± 3 . 1·73	25·1**	3·55
	Dry weight in g.	1·42 ± 3 . 0·15	1·48 ± 3 . 0·48	4·1†	0·12
Roots	Length in cm.	17·7 ± 3 . 0·82	17·1 ± 3 . 0·55	—2·6†	0·60
	Fresh weight in g.	1·62 ± 3 . 0·08	1·84 ± 3 . 0·06	14·2*	2·20
	Dry weight in g.	0·28 ± 3 . 0·09	0·26 ± 3 . 0·10	—8·4†	0·15
Plants grown in small pots (15 plants)					
Stems	Height in cm.	16·5 ± 3 . 0·58	21·5 ± 3 . 0·56	30·5**	6·17
	Fresh weight in g.	12·0 ± 3 . 0·52	16·8 ± 3 . 0·69	40·1**	5·58
	Dry weight in g.	0·49 ± 3 . 0·06	0·72 ± 3 . 0·03	47·8**	3·43
Roots	Length in cm.	14·5 ± 3 . 0·80	19·4 ± 3 . 1·13	33·8**	3·55
	Fresh weight in g.	0·63 ± 3 . 0·04	0·92 ± 3 . 0·08	46·2**	3·22
	Dry weight in g.	0·10 ± 3 . 0·09	0·16 ± 3 . 0·07	60·8†	0·54

Note: Difference t_{48} t_{28}

** highly significant ($P < 0·01$) $> 2·69$ $> 2·76$

* significant ($P < 0·05$) $> 2·01$ $> 2·05$

† not significant

Table 2. Chlorophyll content of leaves and output of carbon dioxide of leaves of *Begonia semperflorens*, the overground parts of which were sprayed with a solution of humus substances of total concentration 300 mg./l. Control plants were sprayed with water

	Pots	Control plants	Plants sprayed with humus solution	Difference in % of control
Chlorophyll content in mg. in 10 g. of fresh weight of leaves.	large	9·850	10·966	11·4
	small	7·780	9·706	24·8
Amount of CO_2 in mg. expired by 15 g. of fresh weight of leaves.	large	5·3	6·9	30·0
	small	5·9	6·9	16·5

Fig. 1. Influence of humus substances applied to the leaves of *Begonia semperflorens* in larger flower pots. Control on the right, plant sprayed with humus on the left.

Fig. 2.

Fig. 3.

Fig. 2. Influence of humus substances applied to the leaves of *Begonia semperflorens* in smaller flower pots. Control on the right, plant sprayed with humus on the left.

Fig. 3. Inhibitory effect of high concentrations and large doses of humus substances applied to the leaves of *Begonia semperflorens* in larger flower pots. Leaves are deformed, side stems develop.

in the large and the small pots stem lengths were increased due to spraying. Increase in length of stems was not the same in all parts: the lower internodes showed greater increases than those formed at a later stage of the plant's growth. Similarly the size of leaf blades (length and breadth) does not increase evenly over the surface but, as shown in fig. 4, with a maximum in areas 4, 5, 6 and 7 of the leaf. From this it may be concluded that the effect of humus substances applied to the plant on the leaves is most intense in the growth of those organs, which at the time of application are in the early stages of tissue development. With the slowing down of the growth of a plant organ its sensitivity to the action of humus substances also decreases. The special sensitivity of meristems is also evident from the reaction to excessive doses of humus substances (fig.3). The more frequent occurrence of side stems in this case indicates that the inhibitory effect of the growing points on the development of axillary buds is influenced and limited.

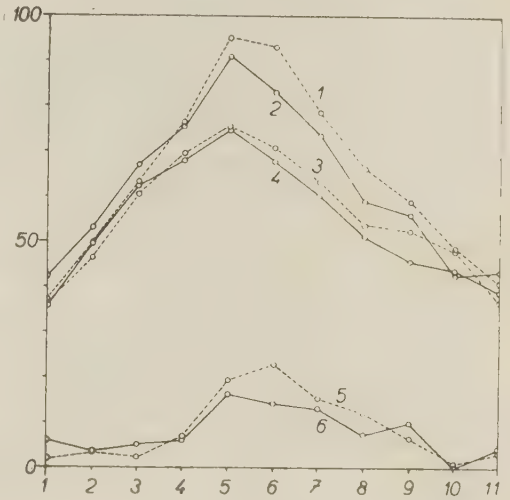

Fig. 4. Influence of spraying with humus substances on length (unbroken line) and breadth (dotted line) of leaves at different levels of *Begonia semperflorens* (large pots). Curve 1·2 — measurements of sprayed leaves, curve 3·4 — measurements of leaves of control. Curve 5 differences in breadth between sprayed leaves and control, curve 6 — differences in length. x: order of leaves on stem from the oldest (1) to the youngest (11), y: measurement in mm.

A further remarkable fact is the difference in the effect of humus substances on plants in large and small flower pots. This phenomenon can also be explained in our experiment by the fact that humus substances improve the utilization of those elements of nutrition which are available to the plant in minimal amounts. Therefore the effect of spraying is greater in the smaller pots, where the source of nutrient is more limited.

The increase in total dry weight of sprayed plants also indicates an increase in intensity of photosynthetic assimilation, that is a more powerful assimilatory apparatus. This is confirmed by the colorimetric determination of chlorophyll content: its value increased for sprayed plants in large pots by 11·4%, in small pots by 24·8%. Thus in this case too humus substances act through spraying in the same way as when they are taken in by the roots (for example Niklewski and Wojciechowski 1947, Wojciechowski 1948, Khristeva 1951).

Besides more intense growth we also observed an increase in intensity of respiration (output of carbon dioxide in the dark). The increase for sprayed plants in large pots was 30% and 16·5% for those in small pots. This effect, too, has already been observed in cases where humus substances were given to plants as a part of the root nutrition (e. g. Khristeva 1951).

In conclusion we may say that humus substances applied to the leaves cause lengthening of the plant organs and increase in their fresh and dry weights. Chlorophyll content and the amount of expired carbon dioxide are also increased. The final effect depends on the period of spraying and on the concentration used. In view of the fact that these effects do not differ from the effects caused by humus substances given as a part of root nutrition, we may assume that they are the result of direct action of humus substances and not of the action of stimulated soil or root microflora, nor to the improvement of physical or physico-chemical character of the soil environment.

A prerequisite for the practical utilization of humus sprays is, however, the extension of experiments to a greater number of plant species, the determination of their sensitivity, suitable concentrations of the solutions and the most suitable time of application.

References

Chizhevski, M. G., Dikusar, M. M.: Rol gumusa i mikroorganizmov v kornevom pitanii vysshikh rasteny v usloviyakh vodnikh i peschanikh kultur. (The role of humus and microorganisms in the root nutrition of higher plants in water and sand cultures.) — Izv. Timiryaz. s-kh. Akad. Moskva **2** : 173—192, 1955.

Godnev, T. N.: Stroenie chlorofilla i metodi ego kolichestvennogo opredelenya. (The structure of chlorophyll and methods of its quantitative determination.) — Minsk 1952.

Khristeva, L. A.: Rol guminovoy kisloti v pitanii rasteny i guminovie udobreniya. (The role of humic acids in plant nutrition and humic fertilizers.) — Tr. pochv. Inst. im. Dokuchaeva **38** : 108—184, 1951.

Niklewski, B., Wojciechowski, J.: Wpływ kwasów próchnicznych na rozwój roślin. (The influence of humic acids on the development of plants.) — Acta Soc. Bot. Pol. **18** : 63—90, 1947.

Prát, S.: Literatura a poznámky o biologickém vlivu humolitů (hlavně kapucínů). (Literature and notes on the biological influence of humolites.) — Cyklostyl. Praha 1955.

Wojciechowski, J.: Porównawcze badania nad wpływem żelaza i związków próchnicznych na rośliny. (Comparative research on the influence of iron and humic compounds on plants.) — Rozpr. Pol. Ak. Umiej. **73**, dz. B: 26—28, 1948.

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Внекорневое применение гумусных веществ

ЗДЕНЕК СЛАДКЫ — ВЛАДИМИР ТИХЫ

Резюме

Некоторые авторы полагают, что гумусные вещества действуют на растение не прямо, а только стимулируют развитие корневой и почвенной микрофлоры, действие которой потом является собственной причиной улучшения роста растения. С целью изучения благоприятного влияния гумусных веществ на рост и метаболизм растения мы исключили их влияние на почвенную и корневую микрофлору, вводя их в растение путем опрыскивания листьев. Одновременно нашей задачей было установить возможность использования благоприятного действия гумусных веществ при опрыскивании растений в практике растениеводства.

На основании предварительных опытов для работ было выбрано растение *Begonia semperflorens* Link et Otto, чувствительно реагирующее на гумусные вещества. Для опрыскивания применялся диализированный экстракт садовой почвы, содержащий гумат натрия с примесью фракции фульвокислот в общей концентрации 300 мг/л. Отдельные дозы, применявшиеся 2 раза в неделю в течение главной фазы роста, составляли 2 мл.

Было установлено, что нанесение гумусных веществ на лист вызывает удлинение органов растения и увеличение их свежего и сухого веса. Повышается и содержание хлорофилла и количество выдыхаемого углекислого газа. Окончательный эффект зависит от времени опрыскивания и от используемой концентрации. Можно полагать, что это действие является результатом непосредственного влияния гумусных веществ, а не влияния стимулируемой почвенной и корневой микрофлоры или улучшения физических или же физико-химических свойств почвенной среды.

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Observation of IEP_M Values of Growing Points of Flower Buds of the Apple during the Period before their Morphological Differentiation

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Souhrn

Byly zjišťovány hodnoty IEP_M u meristematických pletiv vzrostných vrcholů květních pupenů, u floému a xylému letorostů a brachyblastů u tří odrůd jabloní: Průsvitné žluté, Lord Lambourne a Watervlietské.

Řezy 50 μ silné, zhotovené ručním mikrotomem, byly 10 min. fixovány v 70% ethanolu a poté umístěny na 2 hod. do řady pufrovaných (citrátové pufry podle Sörensena) roztoků kyselého fuchsinu a anilinové zeleni (konc. 1 : 10 000), stupňovaných po 0,2 pH. Dále byly řezy přemístěny na půl hodiny do bezbarvých pufrů stejné hodnoty pH.

Za isoelektrickou oblast bylo pokládáno zbarvení pletiv od červenofialového do modrozeleného odstínu. Střední hodnota isoelektrické oblasti byla značena jako IEP_M.

Průběh hodnot IEP_M vzrostných vrcholů pupenů se vyznačoval dvěma fázemi. V první fázi od počátku studia do 9. 7. 1957 zůstávaly hodnoty IEP_M na úrovni 3,0 s maximálními odchylkami 0,1. Ve druhé fázi od 9. 7. do 6. 8. 1958 došlo k posunu hodnot IEP_M směrem k vyššímu pH. Posun však byl dočasného rázu. Po dosažení hranice u odrůdy Průsvitné žluté 3,5, Lord Lambourne 3,6 a Watervlietské 3,3 došlo k posunu hodnot IEP_M na původní úroveň (3,0). V období zpětného posunu hodnot IEP_M k oblasti o nižším pH byl zjištěn počátek morfologické diferenciace vzrostných vrcholů.

U floému a xylému brachyblastů a letorostů mimo podobný posun hodnot IEP_M k oblasti o vyšším pH v období diferenciace vzrostných vrcholů byl zjištěn posun ještě v období zastavení dlouživého růstu na začátku léta.

Summary

1. During the period from June 11 to August 6, 1957 IEP_M values were recorded for the meristematic tissues of growing points of apex buds of brachyblasts and for phloem and xylem of brachyblasts and growing spurs of apple varieties Transparent Yellow, Lord Lambourne and Watervliet.

2. For a period of 7—14 days before the morphological differentiation of growing points of flower buds the shift of IEP_M values towards the area of higher pH was observed. This shift was found both in the meristematic tissues of growing points and in the phloem and xylem of brachyblasts and growing spurs.

3. After reaching a certain limit a shift of IEP_M values back to the area of lower pH at the original level was observed.

Introduction

The development of flower buds of the apple has already been adequately studied from the morphological aspect. The period of the start of morphological differentiation of flower buds has already been established for many varieties (HOŘAVKA 1957) and individual phases of the formation of flower organs have been recorded. Physiological processes taking place in the growing points during the period before their differentiation have, however, been studied only to a limited extent. Since these processes include among others physico-chemical processes, the author turned his attention to following the values of the isoelectric area of growing points of buds and, in addition, recorded the values for the phloem and xylem of brachyblasts and growing spurs of apple trees. In the course of the investigation, however, the extent of the isoelectric area came to involve a varied number of tenths of pH and, therefore, the median value, commonly known in the literature as index IEP_M, was employed to facilitate a comparison of results.

The values of the isoelectric point (IEP) have been mainly determined for micro-organisms and by many authors, from the methodical aspect, for the epidermis of onion *(Allium cepa)* leaves. Some authors have studied the values of IEP in certain plants during growth and development (KONAREV 1948a, b), others have investigated the course of IEP values in different parts of the cell (STRUGGER 1932, DRAWERT 1937 and others). Their results are, however, markedly divergent. From the available literature we know of only one investigation of values of IEP for apple buds (TSELNIKER 1949).

Materials and Methods

IEP_M values, as has been already mentioned, were determined for the meristematic tissues of the growing points of the apex buds of brachyblasts and for phloem and xylem of brachyblasts and growing spurs in three varieties of apple (Transparent Yellow, Lord Lambourne and Watervliet). Sections 50 μ thick, cut by a hand microtome, were fixed with 70% ethanol, which according to ZEIGER (1930) is the most suitable fixative since its causes the least shift of IEP of the specimens studied from the actual values. According to SCHWANTES (1952) other fixatives, such as methanol, sublimate, osmic acid, Carnoy mixture, shift IEP towards higher pH values as compared with 70% ethanol while potassium dichromate, formaldehyde and formaldehyd-ethanol shift it towards lower pH values.

The fixed sections were stained in a buffered solution of acid fuchsine and aniline green. Citrate buffer after SÖRENSEN, with 0·2 pH intervals was used to stabilize the mixture. The actual pH values of the buffers were determined potentiometrically and did not differ by more than $\pm 0{\cdot}03$ pH from the tabular values. Each of the stains used was present in the buffer in concentration of 1 : 10 000. Higher concentrations, as shown by model experiments, shift the IEP_M values towards the area of lower pH.

After fixation, the sections were stained for 2 hours in the given staining mixture and then rinsed for 30 minutes in colourless buffer solutions of the same pH value. The isoelectric area was indicated by staining of the tissues from reddish-violet to bluish-green. As a result of their positive charge the tissues were stained red only by acid fuchsine at lower pH than the lower limit of the isolelectric area, while at higher pH than the upper limit of the isoelectric area they were,

as a result of their negative charge, stained green only by aniline green. The upper and lower limits of the isolelectric area of the tissues were compared with mixtures of the above stains sealed in glass ampules in proportion 1 : 10, 1 : 9 and 9 : 1, 10 : 1. This made it possible to determine the upper and lower limits of the isoelectric area sufficiently accurately and individually at all stages.

In work involving the description of the method of ascertaining IEP there is often no mention of the purity of the stains used. This is a question of first-rate importance and, therefore, we consider it necessary to pay some attention to it.

In general microtechnical practice the stains used, even the fairly pure, alway contain a certain amount of admixture. This impurity, which usually possesses different qualities than the basic stain, has an unfavourable effect on the IEP values obtained. It is therefore necessary as a preliminary measure to verify the purity of the stains by chromatograph paper. So, for example, acid fuchsine contained a certain amount of basic admixture (probably basic fuchsine). Kations of this admixture, after the IEP of the object under study is exceeded, travel by electroadsorption together with the kations of the basic stain (in our case aniline green). This leads to an apparent extension of the isoelectric area and thus also of IEP_M values towards the area of higher pH.

We removed the undesirable basic admixture from acid fuchsine by adsorption with active charcoal repeated several times. Chromatographic control showed that the acid fuchsine was now free of impurity. The aniline green which we used (imported, maker not stated) was very pure and further cleansing was not required.

IEP_M values of the objects mentioned above were determined during the period from June 11 to August 6, 1957 at regular weekly intervals. Values entered on the graphs were always calculated statistically from 4 simultaneous observations. Determination of the significance of differences was made by calculation of the value *t*. Significant differences are marked in the graphs by the letter *S*.

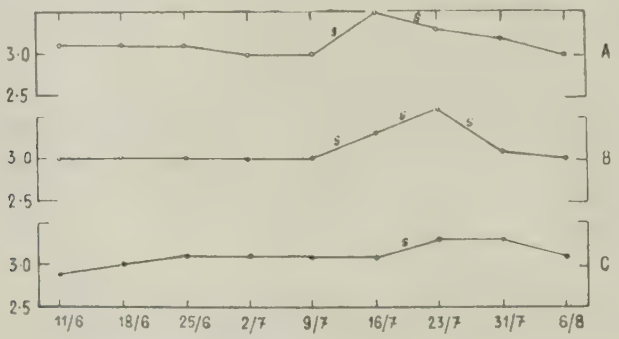

Figure 1. The values of IEP_M of the growing points of the varieties of apple Transparent Yellow (A), Lord Lambourne (B) and Watervliet (C).

Results and Discussion

The course of IEP_M values of the growing points of buds showed two phases during the period of study. In the first phase from the start to July 7, 1957 IEP_M values remained at the level IEP_M 3·0 with maximum deviations of 0·1. In the second phase from July 9 to August 8, 1957 IEP_M values were of quite a different character. With all three varieties there was a shift during this period of IEP_M values towards the area of higher pH (fig. 1). This shift to the area of higher pH was only temporary and after a certain level was reached (for Transparent Yellow 3·5, Lord Lambourne 3·6 and Watervliet

3·3) there was a shift of IEP$_M$ values back to the area of lower pH at the original level. The most important fact, however, in this phase is that in all three varieties, during the period when, following the certain maximum there was a backward shift of IEP$_M$ values to the area of lower pH, the beginning

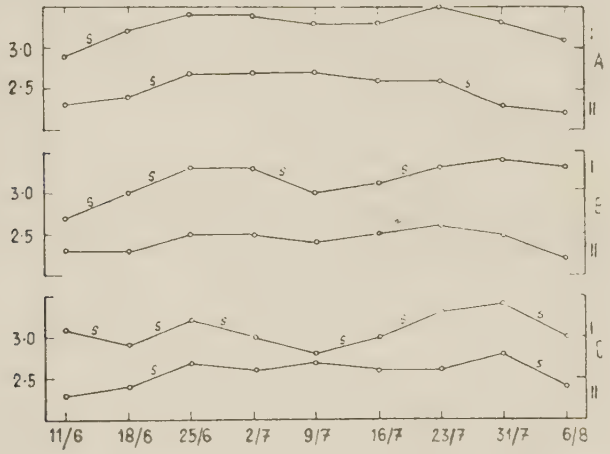

Figure 2. The values of IEP$_M$ of floem (I.) and xylem (II.) of brachyblasts of the varieties of apple Transparent Yellow (A), Lord Lambourne (B) and Watervliet (C).

of the morphological differentiation of the growing points of flower buds was recorded. Thus, in the case of Transparent Yellow the start of this process was recorded on July 23 and for the other varieties, Lord Lambourne and Watervliet, a week later on July 31.

A similar tendency of IEP$_M$ values to shift towards the area of higher pH was also observed in the case of phloem and xylem of brachyblasts and growing spurs. The whole course of IEP$_M$ values for these tissues was further complicated by the fact that in the course of study it was found that there were two maxima of IEP$_M$ values in the area of higher pH (fig. 2 and 3). The first maximum, which was not so marked as the second, was noted in the period of the stoppage of growth in length in early summer (for Transparent Yellow growth in length was halted on June 18 and for Lord Lambourne and Watervliet June 25). The second, more marked maximum of shift in value to the area of higher pH was observed in the period of morphological differentiation of the growing points of flower buds, that is according to variety from July 23 to July 31, 1957.

The established fact of the shift in the IEP$_M$ values of meristematic tissues of growing points in flower buds and of phloem and xylem of brachyblasts and growing spurs, towards the area of higher pH offer us the opportunity to fix beforehand the probable commencement of such an important phenomenon as the differentiation of growing points of flower buds.

The question of whether this phenomenon will be analogous in different years with our varying climatic conditions cannot at this period be answered positively on the basis of our present knowledge. It was, for example, found that the time difference in the start of morphological differentiation of the

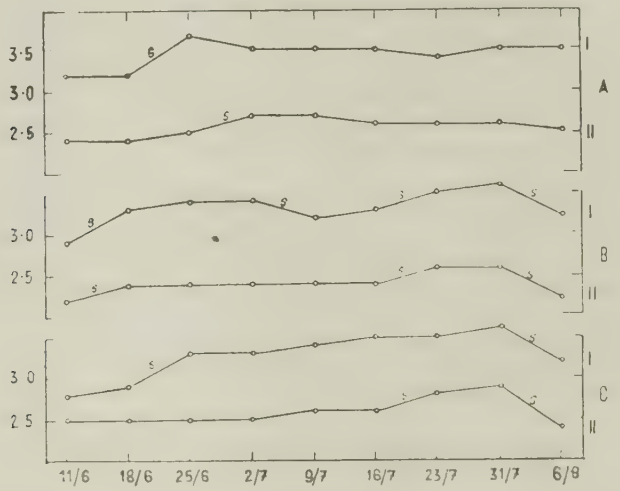

Figure 3. The values of IEP_M of floem (I.) and xylem (II.) of growing spurs of the varieties of apple Transparent Yellow (A), Lord Lambourne (B) and Watervliet (C).

growing points of flower buds for the same variety on one site is 14 days or more. As a result we may expect a time shift of this and other phenomena affecting this process both in the direction of extension and of reduction of this time period. Definite differences can also be expected in varieties with periodic fertility, particularly in years following a good crop, when the setting of flower buds is minimal. In addition, the analogous course of IEP_M value changes in the vegetative and generative organs gives an indication of a mutual relation existing between these organs.

References

Tselniker, J. L.: O fiziologicheskoi differentsirovke tsvetochnikh pochek u jabloni. (On the physiological differentiation of flower buds of apple.) — Dokl. AN SSSR **66** : 281—284, 1949.

Drawert, H.: Das Verhalten der einzelnen Zellbestandteile fixierter pflanzlicher Gewebe gegen saure und basische Farbstoffe bei verschiedenen Wasserstoffkonzentrationen. — Flora **132** : 91—124, 1937.

Hořavka, B.: Diferenciace květních pupenu jabloní. (Fruit-bud differentiation of apple trees.) Čs. biologie (Praha) **6** : 462—465, 1957.

Konarev, V. G.: Vozrastnie izmeneniya v kletkakh rasteniya i izoelektricheskaya tochka protoplasmi. (Changes in plant cells during aging and the isoelectric point of protoplasm.) — Dokl. AN SSSR **59** : 773—776, 1948a.

Konarev, V. G.: Vozrast kletok rasteniya i otnosheniye kletochnikh obolochek k kislim i osnovnim kraskam. (The age of plant cells and the relation of cell walls to acidic and basic stains.) — Dokl. AN SSSR **59** : 983—986, 1948b.

Schwantes, H. O.: Färbungsanalytische Untersuchungen zur Lage des isoelektrischen Punktes der Zellbestandteile in wachsenden Zellen und Geweben. — Protoplasma **41** : 382—414, 1952.

Strugger, S.: Über das Verhalten des pflanzlichen Zellkerns gegenüber Anilinfarbstoffen. — Planta **18** : 561—570, 1932.

Zeiger, K.: Der Einfluss von Fixierungsmitteln auf die Färbbarkeit histologischer Elemente. — Ztschr. wiss. Mikrosk. **47** : 273—293, 1930.

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Исследования величин ИЭТ$_M$ точек роста цветочных почек яблонь в период перед их морфологической дифференциацией

БОРЖИВОЙ ГОРЖАВКА

Резюме

Определялись показатели средней изоэлектрической точки (ИЭТ$_M$) у меристематических тканей точек роста цветочных почек, у флоэмы и ксилемы побегов и коротких плодоносящих веточек трех сортов яблонь: «Налив желтый», Lord Lambourne и Watervliet.

Срезы в 50 μ толщиной, делавшиеся ручным микротомом, фиксировались 10 мин. в 70% этиловом спирте, после чего на 2 часа помещались в ряд буферных (цитратные буферы по Серензену) растворов кислого фуксина и анилиновой зелени (концентрация 1 : 10 000), значение pH которых каждый раз увеличивалось на 0,2. После этого срезы на полчаса помещались в бесцветные буферные растворы при тех же показателях pH.

Изоэлектрической областью считались зоны окраски тканей от краснофиолетового до синезеленого оттенка. Средний показатель изоэлектрической области был обозначен ИЭТ$_M$.

Кривая показателей ИЭТ$_M$ точек роста почек характеризовалась двумя фазами. В первой фазе, от начала исследований до 9/7 1957 г., показатели ИЭТ$_M$ оставались на уровне 3,0 с максимальными отклонениями 0,1. Во второй фазе, с 9/7 по 6/8 1957, наблюдалось смещение показателей ИЭТ$_M$ в направлении к более высоким величинам pH. Но это смещение носило временный характер. После того, как оно у сорта «Налив желтый» достигло предела 3,5, у Lord Lambourne 3,6, а у Watervliet — 3,3, наблюдалось смещение показателей ИЭТ$_M$ до первоначального уровня (3,0). В период обратного движения показателей ИЭТ$_M$ к области с более низким pH было отмечено начало морфологической дифференциации точек роста.

У флоэмы и ксилемы плодоносящих веточек и побегов, кроме такого смещения показателей ИЭТ$_M$ по направлению к области с более высоким pH в период дифференциации точек роста, было отмечено смещение еще в период остановки роста в длину в начале лета.

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The Influence of Gibberellic Acid on the Growth of Overground Parts and Roots of Wheat, Lettuce and Oats

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Souhrn

Byl sledován vliv různých koncentrací kyseliny giberelové (GA) na klíční rostliny pšenice, salátu a ovsa. Kultivace byla provedena v klimatisační komoře na Petriho miskách v Knopově živném roztoku s přidáním GA. V pokusech byla hodnocena čerstvá váha a sušina nadzemních částí a kořenů a biometricky zpracovány údaje o jejich délce. Byla rovněž sledována dynamika délky přírůstků nadzemních částí a kořenů, jakož i údaje o čerstvé váze a sušině u salátu. V pokusech byla zjištěna průkazná stimulace délky nadzemních částí ve všech použitých koncentracích (0,1—100 μg GA/ml) a průkazná inhibice délky kořenů u koncentrací 1 až 100 μg GA/ml.

Výjimkou byl oves, u něhož byla zjištěna stimulace délky kořenů při koncentraci 10 μg GA/ml. Čerstvá váha byla zvýšena pouze u nadzemních částí pšenice (100 μg GA/ml) a u salátu. Stejný obraz podávají i údaje o sušině. Čerstvá váha i sušina kořenů u pšenice a ovsa byla aplikací GA snížena nebo se nezměnila. Dynamické sledování stimulace nadzemních částí vyvolané GA u salátu ukazuje, že rozdíl, který je zjištěn čtvrtý den, během další kultivace prakticky nevzrůstá. V daných experimentálních podmínkách je tedy základní stimulační efekt vyvolán již mezi 3—4 dnem kultivace klíčních rostlin.

Summary

1. The influence of varying concentrations of gibberellic acid on seedlings of wheat, lettuce and oats (up 10 days old) was observed and optimal concentration of 10 to 100 μg GA ml. was established. Biometric evaluation of the experiments showed significant stimulation of the overground parts with all concentrations used (0·1 to 100 μg. GA/ml.) and significant inhibition of the roots with concentrations 1 to 100 μg. GA/ml.

2. For lettuce only the concentration 10 μg. GA ml. was used. In this case, too, increase in length of overground parts was significant and inhibition of root growth was observed. For oats the stimulating effect of GA was recorded for both overground parts and roots.

3. Fresh weight of wheat was stimulated only by concentrations of 100 μg. GA/ml. In the case of lettuce (where values of fresh and dry weight were taken for the whole plant) we found a marked stimulation of fresh weight and a somewhat smaller increase in dry weight.

4. Observation of the dynamics of growth in length of overground parts and roots and increase or fresh and dry weight in lettuce showed a mutual relation between the values recorded in control and experimental plants in the period of 4 to 10 days following germination.

Introduction

In recent years the interest of plant physiologists in gibberellic acid (further GA) has been growing. This new growth stimulator has a particularly marked influence on the growth in length of overground parts of higher plants. Although there is a considerable volume of work in the world literature dealing with the evaluation of growth changes following the application of GA (a more detailed analysis of the literature is given in the discussion), the conclusions drawn are not always consistent. In undertaking the physiological study of the effect of GA we were obliged to verify some of the fundamental facts anew. We present for the most part the results of experiments with wheat, lettuce and oats, for these plants proved to be the most suitable and most sensitive as model material. The aim of the experiments was to acquire data on the growth of overground parts and roots of seedlings after the application of GA and to determine the relation between the concentration used and the stimulatory or inhibitory effects produced. We recorded both the lengths of overground parts and roots and also the fresh and dry weights. In addition we endeavoured to follow the dynamics of the growth of lettuce following the action of GA.

Material and Methods

We used the following varieties in our experiments: wheat, Hodonínská holice (1956 harvest); lettuce, Stupický kamenáč (1955 harvest); oats, Český žlutý (1956 harvest). The plants were grown in Petri dishes (14 cm. diameter). Number of plants in each group: wheat and oats 50 plants of each, lettuce 200 plants. As the basic nutrient solution we used Knop's solution in distilled water with A—Z solution of microelements after Hoagland. We applied GA dissolved in Knop's solution (with A—Z solution) in concentrations as given later in the text. We cultivated the plants in climatic chambers at temperatures varying between 18—25° C (registered by thermograph). Lighting in the chambers was by electric bulbs and mercury lamps with an intensity of 2000 lux at the level of the Petri dish for 12 hours a day. Every third day we renewed the solutions and rinsed the roots with distilled water. At the first exchange of solutions we replaced the lids of the Petri dishes by three-litre beakers and removed the ungerminated seeds. Results of the experiment were assessed on the tenth day. We evaluated the results biometrically for 20 plants by measuring the heights of overground parts (from the grain to the leaf tips) and the lengths of all roots. We also described the habitus of the plants. For all plants we determined fresh and dry weights in the usual way. In experiments where the effect of GA on lettuce was followed dynamically we proceeded in a similar way. We took specimens at intervals as given in the tables and graphs. We repeated the experiments three times (with the exception of the experiment for dynamic observation of lettuce after application of GA). Since we obtained similar results we present only one experiment as an example.

The authors wish to express their thanks to Miss J. Veverková for her technical assistance.

Table 1. Influence of GA on the length of overground parts of wheat

Variant	n	$\bar{x}$	$\Sigma (\bar{x} - x)^2$	P				
				Control	0·1 μg	1·0 μg	10·0 μg	100·0 μg
Control	20	220	4403	—	< 0·01	< 0·01	< 0·01	< 0·01
0·1 μg. GA/ml.	20	267	5343	< 0·01	—	< 0·01	< 0·01	< 0·01
1·0 μg. GA/ml.	20	292	5854	0·01	< 0·01	—	< 0·01	< 0·05
10·0 μg. GA/ml.	20	313	6269	< 0·01	< 0·01	< 0·01	—	N
100·0 μg. GA/ml.	20	287	6113	< 0·01	< 0·01	< 0·05	N	—

N = difference not significant
P = degree of significance

Results

We determined the effect of varying concentrations of GA on the linear growth of overground parts. Results are given in fig. 1 and in table 1. Data were taken at the phase of the second leaf. We found narrower, awl-shaped leaves and slight chlorosis in the experimental plants as compared with the control. Reduction of turgor was also noticeable in the experimental plants, causing bending of the leaves. From the graphs and tables it can be seen that with a concentration of 0·1 μg. GA/ml. there is already a significant incerease in length of overground parts of wheat. The optimal concentration lies between 10 and 100 μg./ml. This conclusion is confirmed by another of our experiments

Table 2. Influence of GA on the length of roots of wheat

Variant	n	$\bar{x}$	$\Sigma (\bar{x} - x)^2$	P				
				Control	0·1 μg.	1·0 μg.	10·0 μg.	100·0 μg.
Control	20	366	7326	—	N	< 0·05	< 0·01	< 0·01
0·1 μg. GA/ml.	20	346	6916	N	—	N	< 0·01	< 0·05
1·0 μg. GA/ml.	20	318	6362	< 0·05	N	—	< 0·05	N
10·0 μg. GA/ml.	20	285	5698	< 0·01	< 0·05	< 0·05	—	N
100·0 μg. GA/ml.	20	286	5735	< 0·01	< 0·05	N	N	—

N = difference not significant
P = degree of significance

(not given here), in which we used a concentration of 50 μg. GA/ml. This concentration gave rise to a greater increase in length than did that of 10 μg./ml. In evaluating the lengths of roots we observed an opposite effect of GA, that is inhibition. Results are given in fig. 2 and table 2. Stimulation of

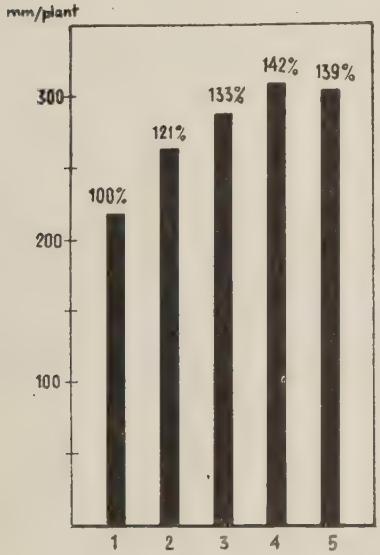

Fig. 1. Average length of overground parts of wheat plants following application of GA. 1 — control, 2 — 0·1 μg. GA/ml., 3 — 1·0 μg. GA/ml., 4 — 10 μg. GA/ml.

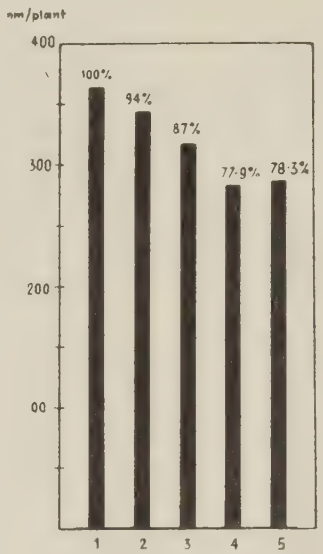

Fig. 2. Average length of wheat roots following application of GA. 1 — control, 2 — 0·1 μg. GA/ml., 3 — 1 μg. GA/ml., 4 — 10 μg. GA/ml., 5 — 100 μg. GA/ml.

root growth was not found in a single case. The difference between the 0·1 μg GA/ml. concentration and the control is, however, insignificant. We were interested to see whether a further reduction in concentration of GA might not give rise to a stimulatory effect on the roots. Experiments, the results of which are not given here in detail, showed that with further reduction of GA concentration below 0·1 μg./ml. (concentrations 0·1, 0·01, 0·001 and 0·0001 μg. GA/ml. were tried) the solutions do lose their inhibitory influence, but on the other hand in no case did we observe any stimulatory action.

The results of experiments with lettuce, in which we used only one concentration (10 μg./ml.), are of a similar character to those with wheat (table 3). The degree of stimulation of the overground parts and the degree of inhibition of the roots was, however, considerably higher here. This was also evident from the habitus of the experimental plants, which were chlorotic, had very elongated hypocotyls and markedly extended leaves. The dynamic observation of growth of overground parts and roots (fig. 3) showed that, in spite of some fluctuations, the degree of stimulation remains in general steady with a tendency to reduction of differences.

Table 3. Influence of GA on lettuce plants

Variant	Length of overground parts in mm.		Length of main root in mm.		Fresh weight of 1 plant in mg.		Dry weight of 1 plant in mg.	
Control	27·8	100%	31·0	100·0%	34·4	100%	1·49	100%
10 μg. GA/ml.	57·3	206%	19·4	62·5%	54·7	158%	1·69	113%

Table 4. Influence of GA on oats plants

Variant	Length of overground parts of 1 plant in mm.	Length of roots of 1 plant in mm.	Overground part		Roots	
			Fresh weight of 1 plant in mg.	Dry weight of 1 plant in mg.	Fresh weight of 1 plant in mg.	Dry weight of 1 plant in mg.
Control	100·0% 191·50	100·0% 322·0	100·0% 152·5	100·0% 12·45	100·0% 52·5	100·0% 3·68
10 μg. GA/ml.	139·5% 267·50	109·5% 363·0	98·3% 157·5	98·3% 12·25	98·3% 50·0	90·0% 3·31

Table 5. Table of fresh and dry weights of wheat

Variant	Overground part				Roots			
	Fresh weight of 1 plant in mg.		Dry weight of 1 plant in mg.		Fresh weight of 1 plant in mg.		Dry weight of 1 plant in mg.	
Control	157·0	100·0%	17·92	100·0%	44·0	100·0%	5·37	100·0%
0·1 μg GA/ml.	140·5	89·0%	16·79	93·8%	37·5	85·5%	4·28	79·7%
1·0 μg. GA/ml.	146·0	93·0%	17·63	98·5%	44·5	101·0%	4·38	81·5%
10·0 μg. GA/ml.	150·0	95·6%	18·45	103·0%	42·0	95·5%	4·27	79·6%
100·0 μg. GA/ml.	175·0	111·3%	20·57	114·5%	33·0	75·0%	4·20	79·2%

With oats using one concentration of 10 μg. ml. we obtained the same stimulation of overground parts as with wheat. In contrast to the results with wheat and lettuce we found here, however, a significant stimulation of the roots (table 4).

Comparison of fresh and dry weights of experimental and control plants of wheat is given in table 5. The fresh weight of overground plants was, with the exception of cases where concentration 100 μg. ml. was used, slightly reduced. A similar reduction, of a greater degree, was found from the dry weight results. In the case of roots fresh and dry weights were reduced in all plants.

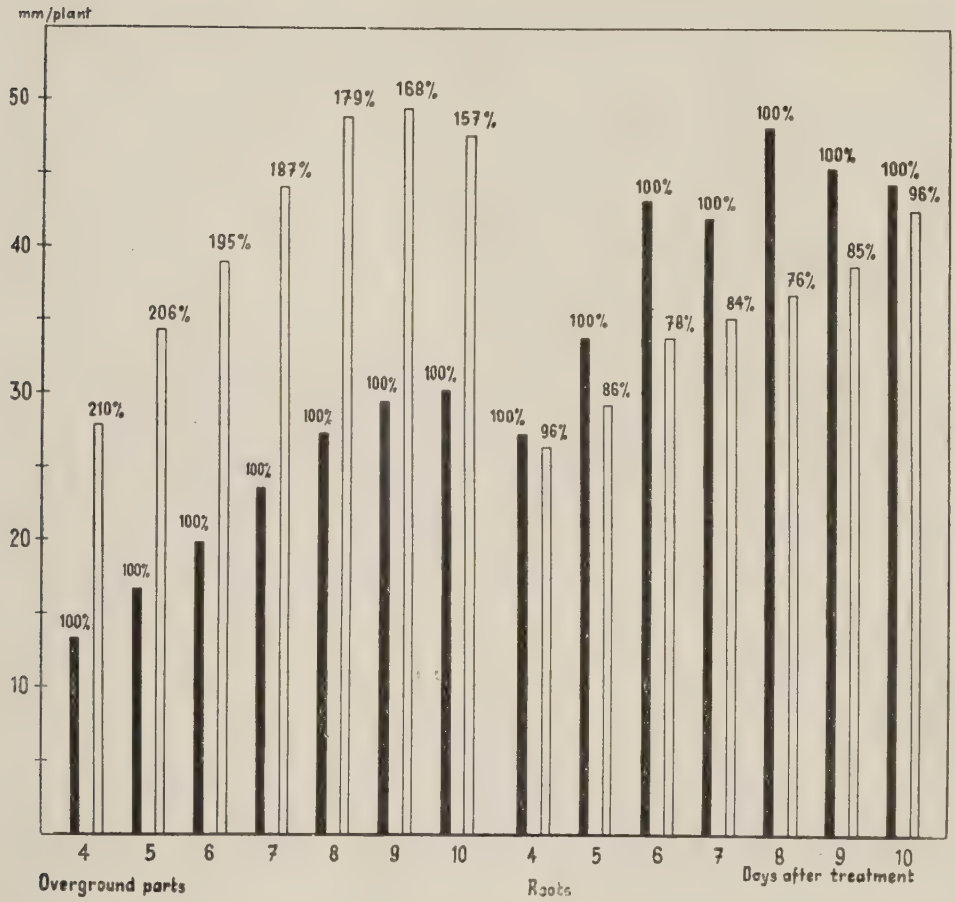

Fig. 3. Dynamic study of the average length of overground parts (left) and roots (right) of lettuce following application of GA.
■ — control, □ — GA

In table 3 fresh and dry weights for lettuce are recorded. We evaluated the fresh and dry weights of whole plants. In contrast to wheat we found with a concentration of 10 μg./ml. a stimulation of both these values. The fresh weight was stimulated more than the dry. In experiments recording the dynamics of fresh and dry weight values (with the same concentration) mutual fluctuation of controls and experimental plants was again evident (fig. 4 and 5). The degree of stimulation (for overground parts and roots) remained nearly unchanged during the whole course of the experiment. For roots there is greater inhibition of dry than of fresh weight and the values show considerable deviations.

Results for fresh and dry weights of oats resemble those for wheat (table 4).

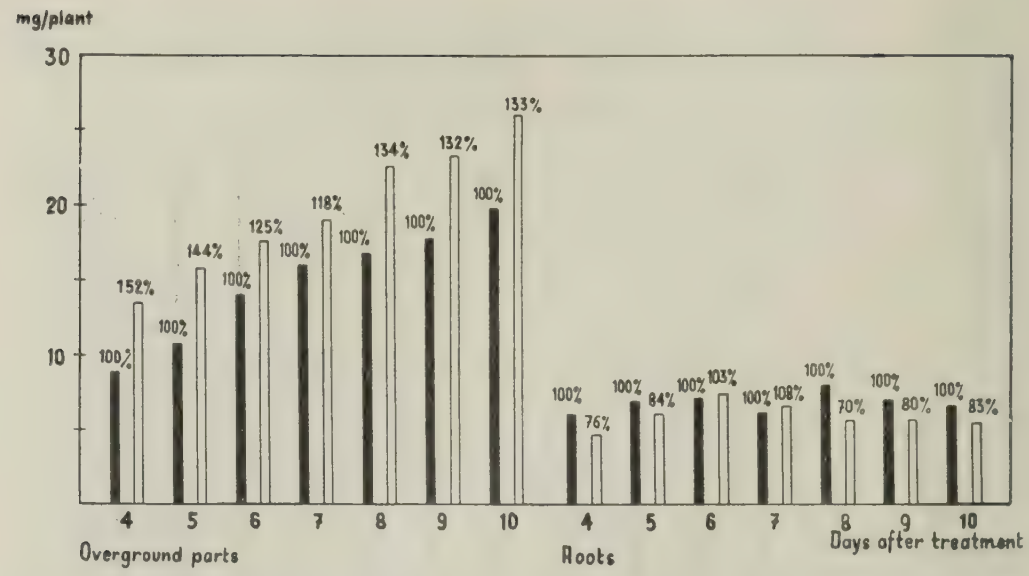

Fig. 4. Dynamic study of the average fresh weight of overground parts and roots of lettuce following application of GA.
■ — control, □ — GA

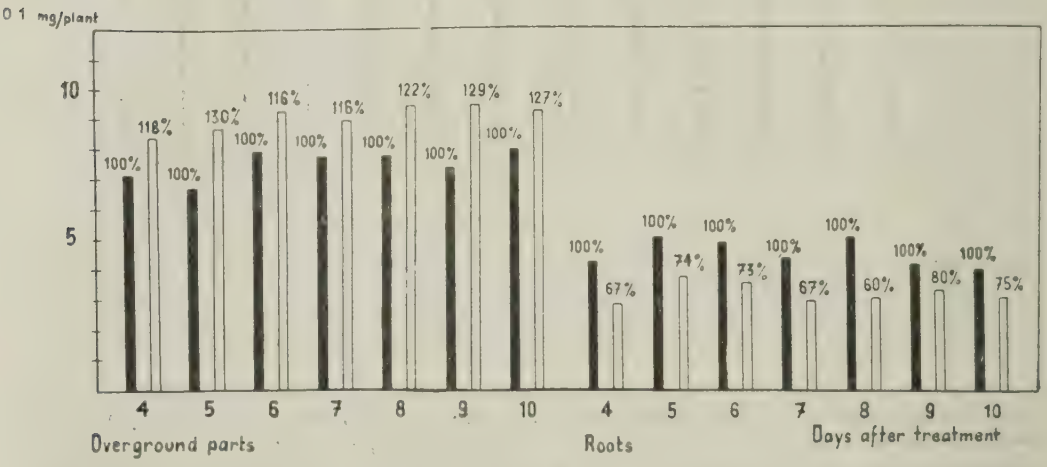

Fig. 5. Dynamic study of the average dry weight of overground parts and roots of lettuce following application of GA.
■ — control, □ — GA

Discussion

In our experiments we have confirmed the results of many authors (Brian et al. 1954, Brian and Hemming 1955, Marth et al. 1956, Stowe and Yamaki 1957, Wittwer et al. 1956, Wittwer and Bukovac 1957, Yabuta and

Hayashi 1939) with regard to the considerable stimulatory effect of GA in increasing the length of overground parts.

The question of the influence of GA on root growth has not as yet been definitely solved. Kato (1956) denies the influence of GA on root growth altogether; on the other hand, Whaley and Kephart (1957) demonstrated a stimulatory influence of GA on maize roots in vitro. Wittwer and Bukovac (1957) call attention to a much greater stimulatory effect of GA on overground parts than on roots. In the experiments of Brian et al. (1954) the effect of GA on roots was dependent on the method of application. When GA was applied in nutrient solution root growth was inhibited, when it was applied in lanoline paste to the leaves root growth was stimulated. This conclusion was, however, based only on the dry weight of roots. In our experimental conditions inhibition of the roots was apparent not only in the fresh and dry weights, but above all in the length of the roots. Under our conditions of cultivation it might be possible to explain the inhibition by the possibility of the development of a local concentration of GA in the roots, that is by a kind of local overdosing. On the other hand, however, even with reduced concentration a stimulatory effect of GA on the roots was never observed and it is possible that a quite different type of reaction from that of the overground parts is involved. (In this connection the analogical situation found in the study of the effect of auxin on roots should not be forgotten.) An important part with regard to the inhibition of roots and overground parts is also clearly played by significant specific differences in reaction to the action of a given stimulator (e. g. in our experiments with oats stimulation of root length was obtained with application of 10 μg. GA/ml.). Lack of unanimity in the literature is also caused by the choice of different criteria for the evaluation of the same phenomenon. As we have already mentioned, roots are usually judged only by fresh and dry weights (Brian et al. 1954, Wittwer et al. 1956); our measurements of length are practically the first to appear in this connection. We consider the weak inhibitory effect of higher concentrations of GA on roots and their ineffectiveness at lower concentrations to be a common phenomenon, noted frequently in the literature (Brian et al. 1954, Stowe and Yamaki 1957, Wittwer et al. 1956). In the case of oats a special "resistance" of the roots of the variety used is evidently involved.

The stimulation generally noted in the literature (Brien et al. 1954, Brian and Hemming 1955, Stowe and Yamaki 1957, Wittwer et al. 1956, Gibberellic acid, Plant Protection Ltd, Fernhurst Research Station, Great Britain, 1957) of the fresh ad dry weights of the overground parts following application of GA, was confirmed in our experiment only in the case of lettuce, and with the highest concentration, for wheat (100 μg. GA/ml.). In the other cases for the majority of the concentrations we did not find this increase with wheat and oats. It is, however, necessary to take into consideration the different methods of application. Our method, i. e. cultivation in nutrient solution with addition of GA, is not so often used and the differences between our results and the majority of those in the world literature may perhaps be due to this circumstance. As has already been mentioned, the experimental wheat plants showed reduced turgor and were slightly chlorotic at the end of the experiment. The low light intensity with relatively high temperature and insufficient flow

of air to the plants must also be taken into consideration. These are factors which affected primarily the quick growing experimental plants (plants after application of GA).

References

Brian, P. W., Elson, G. W., Hemming, H. G., Radley, M.: The plant-growth promoting properties of gibberellic acid a metabolic product of the fungus Gibberella fujikuroi. — J. Sci. Food Agr. **5** : 602—612, 1954.

Brian, P. W., Hemming, H. G.: The effect of gibberellic acid on shoot growth of pea seedlings. Physiol. Plant. **8** : 669—681, 1955.

Kato, J.: Effect of gibberellin on elongation, water uptake and respiration of pea stem sections. — Science **123** : 1132, 1956.

Marth, P. C., Audia, W. V., Mitchell, J. W.: Effects of gibberellic acid on growth and development of plants of various genera and species. — Bot. Gaz. **118** : 106—111, 1956.

Stowe, B. B., Yamaki, T.: The history and physiological action of the gibberellins. — Ann. Rev. Plant Physiol. **8** : 181—216, 1957.

Whaley, W. G., Kephart, J.: Effect of gibberellic acid on growth of maize roots. — Science **125** : 234, 1957.

Wittwer, S. H., Bukovac, M. J., Weller, L. E., Sell, H. M.: Some effects of gibberellic acid on plant growth and metabolism. — Plant Physiol. **31** : (Suppl.), 1956.

Wittwer, S. H., Bukovac, M. J.: Gibberellins new chemicals for crop production. — Quart. Bull. Agric. Exp. Sta. Mich. East Lansing **39** : 469—494, 1957.

Yabuta, T., Hayashi, T.: Biological studies on bakanae fungus of rice. 3. Physiological action of gibberellin on the plants. — J. Agr. Chem. Soc. (Japan) **15** : 403—413, 1939.

Gibberellic acid, Plant Protection Limited, Fernhurst Research Station, Great Britain, 1957.

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Влияние гиббереллловой кислоты на рост надземных частей и корней пшеницы, салата и овса

ЯН КРЕКУЛЕ — ЯРОСЛАВ УЛЛМАНН

Резюме

Исследовалось влияние различных концентраций гиббереллловой кислоты (ГК) на прорастание семян пшеницы, салата и овса. Культивация производилась в люминостате на чашках Петри в питательном растворе Кнопа с прибавлением ГК. В опытах оценивались свежий вес и вес сухого вещества надземных частей и корней и биометрически обрабатывались данные их длины. Исследовались также динамика роста надземных частей и корней, а также показатели свежего и сухого веса салата. Было доказано стимулирующее действие всех применявшихся концентраций (0,1—100 μг ГК/мл) на длину надземных частей и угнетающее действие на длину корней в концентрациях 1—100 μг ГК/мл.

Исключение составлял овес, у которого стимулирующее действие на длину корней наблюдалось при концентрации 10 μг ГК/мл. Свежий вес оказывался погыщенным только у надземных частей пшеницы (при 100 μг ГК/мл) и у салата. Такую же картину дают и показатели сухого веса. Свежий и сухой вес корней пшеницы и овса после применения ГК понижался или оставался неизменным. Исследования динамики стимулирования роста надземных частей применением ГК у салата показывают, что разница, отмеченная на 4-ый день, в течение дальнейшей культивации практически не увеличивается. Таким образом, в данных условиях опыта основной стимулирующий эффект наблюдается уже 3—4-ый день культивации прорастающих семян.

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Die Bioteknose beim Apfelbaum

Rudolf Řetovský

Biologisches Institut der Akademie der Wissenschaften, Praha

Eingegangen den 3. Feber 1958

Souhrn

Autor nacházel často v létě ve skladovaných plodech jabloně odrůdy Ontario semenáče. Tento zjev zvaný bioteknosa pozoroval autor také v předjaří na plodech odrůdy Wealthy. Proto sledoval soustavněji rychlost zezelenání a růst embryonálního kořínku isolovaných zárodků jabloně odrůdy Průsvitné žluté (letní), Wealthy (podzimní) a Ontario (zimní odrůda), a to během skladování plodů.

Exstirpovaná embrya na vatě v Petriho miskách byla pěstována po dobu 7 dní v definovaných podmínkách tepelných a světelných. Výsledky pokusů byly zpracovány statisticky analysou rozptylu. Rychlost zezelenání děloh a intensita růstu klíčního kořínku závisí na odrůdě a době skladování (stáří plodu). Každá ze studovaných odrůd má významné období, v němž isolovaná embrya ze skladovaných plodů jeví vysokou životnost (neobyčejně rychle klíčí, dělohy rychle zezelenají a embryonální kořínek se rychle prodlužuje). Zárodky těchto vlastností, stejně jako semenáče nalezené přímo v plodech, rostou po přesazení do země hned a intensivně. Krátká doba pěstování isolovaných zárodků (7 dní) umožňuje provést výběr nejživotnějších zárodků.

Autor se domnívá, že pozorované zjevy se mohou využít v ovocnářském výzkumu.

Zusammenfassung

Bei drei Sorten von verschiedener wirtschaftlicher Qualität, Klarapfel, Wealthy und Ontario, wurde während der Fruchteinlagerung die Vitalität exstirpierter Keime untersucht, und zwar nach dem Grad der Grünung der Kotyledonen, nach der Wachstumsgeschwindigkeit der isolierten Keimlinge und nach der Wachstumsintensität der Keimwurzeln. Die Beziehungen dieser beobachteten Charakteristika wurden mittels Faktorenanalyse ausgewertet und mit der als Bioteknose bezeichneten Erscheinung in Verbindung gebracht.

Einleitung

Als Bioteknose wird die Keimung des Samens innerhalb der Frucht bezeichnet (endokarpe Keimung). Quellen zur Geschichte dieser Bezeichnung führt Ulbrich (1928) an. Velenovský (1910) erwähnt die Viviparie, ist sich aber dessen bewusst, dass man unter dieser Bezeichnung biologische Vorgänge beschreibt, die keinen innern Zusammenhang aufweisen. Weder Crocker und Barton (1953) noch Kobel (1954) erwähnen die Bioteknose. Němec (1944, S. 127) schreibt: „Ausnahmsweise wurde eine Keimung innerhalb des Apfels beobachtet, aus dem Achse und Wurzel des Keimlings herausragten (Frenzel, 1877)". Mičurin (1953) beschreibt das Vorkommen von Keimlingen in den Früchten vom Kernobst. Keimlinge innerhalb der Früchte (Bioteknose) kommen bei verschiedenen Pflanzenarten vor (Němec 1941). Passecker (1937) fand sie hauptsächlich in den Früchten amerikanischer Wintersorten des Apfels und beobachtete, dass die

Kerne solcher Sorten ungewöhnlich rasch — explosiv — keimen. Derselbe Autor betrachtet die Lagerung der Früchte als natürlichste Art der Stratifikation der Kerne und empfiehlt die Frühjahrsaussaat von Kernen ausgewählter Früchte. Zwischen der physiologischen Vollreife der Frucht und der Keimung der Kerne besteht tatsächlich eine Beziehung (Bělochonov 1953), die in der Praxis die Qualität der Kerne bestimmt.

Der Übergang der Kerne von der Keimruhe zur Keimreife wird mit Veränderungen der dynamischen Gleichgewichtszustände zwischen Inhibitoren und Stimulatoren erklärt (Audus 1953).

Die vorliegende Arbeit befasst sich mit dem Wachstum isolierter Keime in Abhängigkeit von Sorte und Alter der Frucht.

Material und Methodik

Die Untersuchungen wurden an Früchten der Apfelsorten Klarapfel (Sommersorte), Wealthy (Herbstsorte) und Ontario (Wintersorte) aus der Ernte 1956 vorgenommen. Die Früchte wurden von elfjährigen Bäumen in Střížovice bei Turnov geerntet und im Keller bei einer in den Wintermonaten zwischen 4° und 6° schwankenden Temperatur eingelagert. Die Wintersorten erhielten sich unter diesen Bedingungen bis August 1957.

Die Keimschnelligkeit (Keimenergie) der exstirpierten Keimlinge wurde an Petrischalenkulturen mittels der von Zelinková, Hořavka und Řetovský (1951) beschriebenen Methode untersucht. Die Kulturen waren in einer Box bei einer zwischen 21° bis 23° C regulierten Temperatur untergebracht und von 10 Leuchtstoffröhren (Tesla 25 W B VC) bestrahlt, deren Beleuchtungsstärke etwa 85 cm von der Lichtquelle entfernt in der Höhe der Petrischalen ungefähr 1750 Lux betrug. Die Kulturen der isolierten Keimlinge wurden einer Photoperiode von 12 Stunden Licht und 12 Stunden Dunkel ausgesetzt. Die Versuche dauerten von September 1956 bis Juli 1957.

Nach 7 Tagen wurde der Grünungsgrad der Keimblätter und die Länge der Keimwurzeln bestimmt. Als Nullstufe der Grünung galt der Zustand beider, weisser Keimblätter, als Stufe 10 die Anwesenheit vollkommen grüner Keimblätter. Die Länge der Keimwurzeln ist mit der Genauigkeit auf 1 Millimeter angegeben; Wurzeln kürzer als 1 mm, wurden daher 0 mm lang klassifiziert.

Die Beziehungen zwischen Zeitpunkt der Fruchteinlagerung, Grünungsgrad der Keimblätter und Distribution in den einzelnen Variationsklassen der Grünung sind in Diagrammen dargestellt, aus denen sich die Distributionsfunktion und alle anderen für die Auswertung der Versuche notwendigen Charakteristika ablesen lassen. Auf der y-Achse ist der Grünungsgrad der Keimblätter, auf der x-Achse das Alter der gelagerten Früchte in Monaten und auf der z-Achse die Distribution in den einzelnen Variationsklassen angegeben. Die Wiedergabe tabellarisch angeordneter Zahlenübersichten zur Berechnung der Testungsmerkmale (Abb. 1, 2, 3) erübrigt sich daher.

Die Wachstumsintensität der Keimwurzel in den ersten 7 Tagen wurde in Abhängigkeit von Sorte, Alter der Frucht und Grünungsgrad mittels Streuungsanalyse gewertet. (Die Methodik siehe bei Beneš und Likeš 1957, Myslivec 1957.)

Ergebnisse und Diskussion

Die Grünungsschnelligkeit der Keimblätter isolierter Keimlinge und die Wachstumsintensität der Keimwurzel lassen sich gut als Gradmesser für die Vitalität der Keimlinge verwenden, also für die Schnelligkeit, mit der sie in Wechselbeziehung zu Milieu und Abhängigkeit von diesem eintreten. Dies wird auch durch die Praxis bestätigt. Schnell (explosiv) wachsende Keimlinge wachsen nach ihrer Einbringung in die Erde sofort und auch weiterhin schnell. Nach drei Monaten erreichen die Stämme Höhen bis zu einem halben Meter.

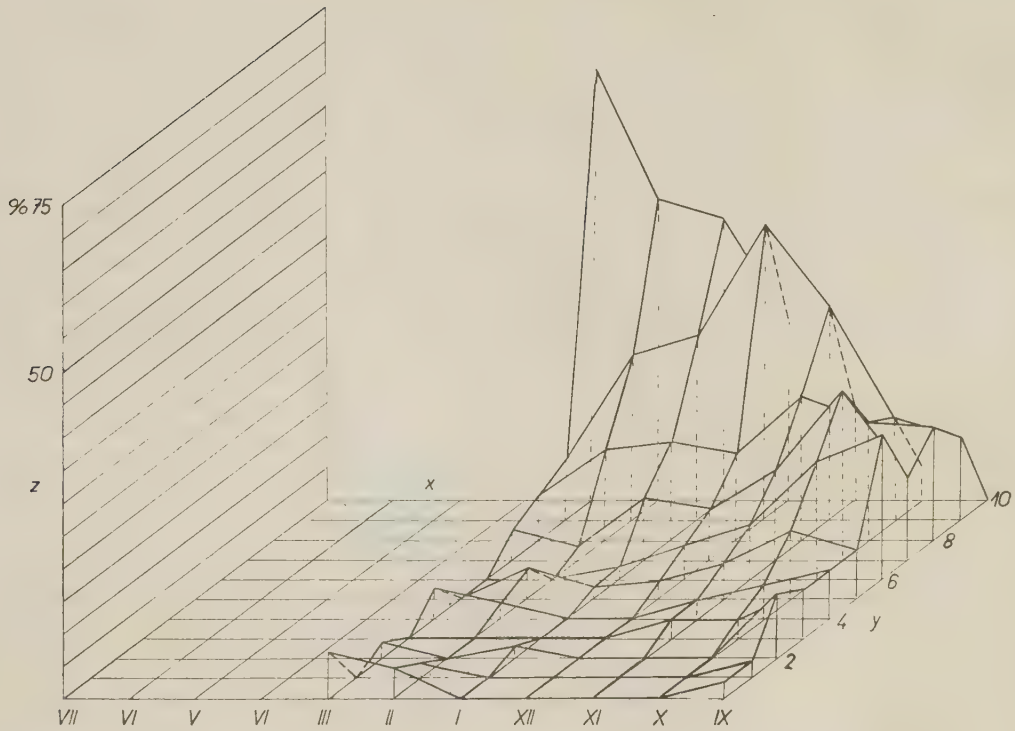

Abb. 1. Schnelligkeit der Grünung exstirpierter Keimlinge aus den Kernen der Apfelsorte Klarapfel in verschiedenen Monaten während der Lagerung. Auf der x-Achse sind die Monate, auf der y-Achse die Grünung der Keimblätter und auf der z-Achse die Distribution in den einzelnen Grünungsstufen aufgetragen. Zeichnung J. Veverková

Auf die Frage, ob bei isolierten Keimlingen von einer Keimung gesprochen werden kann oder nicht, geht die Arbeit von Petrů und Řetovský (1956) theoretisch ein.

Aus den Diagrammen ist zu ersehen, dass bei jeder Sorte ein bestimmtes Stadium vorkommt, in dem die isoliteren Keimlinge fähig sind, rasch zu grünen und zu wachsen. Ein solches Stadium kann sogar bei der Sommersorte Klarapfel beobachtet werden, und zwar im Vorfrühling (März), wenn die eingelagerten Früchte allerdings bereits ungeniessbar sind und die Kerne vor der Exstirpation desinfiziert werden müssen. (Bewährt hat sich eine 15 Minuten lange Desinfektion mit Wasserlösung von Chloramin von ungefähr 1% aktivem Chlorgehalt.) In den Früchten dieser Sommersorte wurden vom Verfasser Keimlinge nicht gefunden, doch nimmt er an, dass es wahrscheinlich auch hier zur Bioteknose kommen könnte, wenn es gelin gen würde, die Früchte bis in den Vorfrühling in gutem Zustand zu erhalten.

Die Vitalität der aus der Apfelsorte Wealthy isolierten Keimlinge ist zu Beginn der Lagerung verhältnismässig schwach, steigt aber mit dem Alter

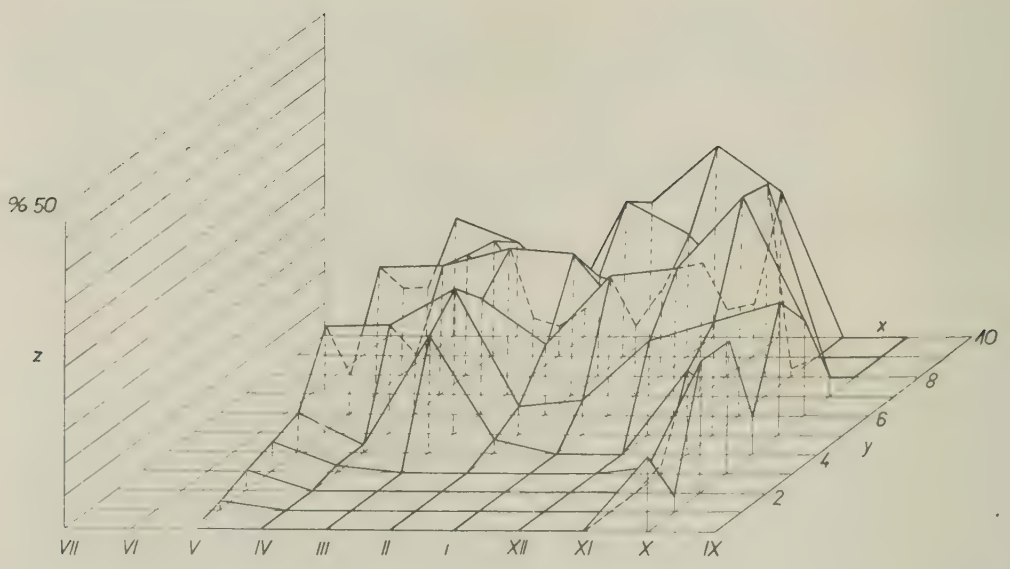

Abb. 2. Schnelligkeit der Grünung exstirpierter Keimlinge der Apfelsorte Wealthy. Bedeutung der Koordinaten wie bei Abb. 1. Zeichnung J. Veverková

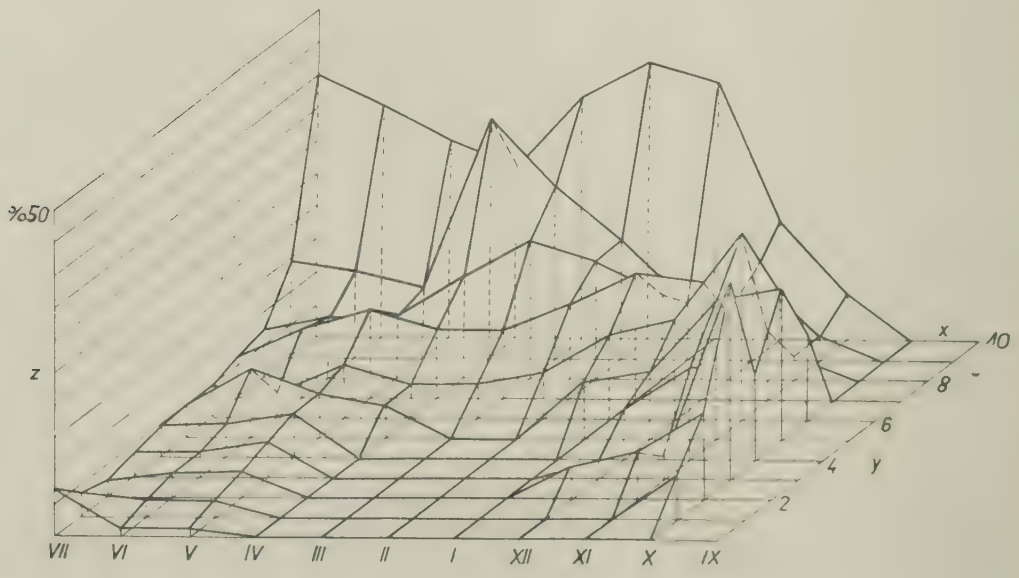

Abb. 3. Schnelligkeit der Grünung exstirpierter Keimlinge der Apfelsorte Ontario. Bedeutung der Koordinaten wie bei Abb. 1. Zeichnung J. Veverková

der Früchte und erreicht ihr Maximum ungefähr im Jänner. Vom März an fand der Verfasser in den Früchten regelmässig vereinzelte Keimlinge oder zumindest angekeimte Kerne. Zu diesem Zeitpunkt sind aber die Früchte dieser Herbstsorte bereits ungeniessbar.

Bei der Sorte Ontario sind zwei Maxima der Vitalität isolierter Keimlinge zu verzeichnen: das Hauptmaximum in der zweiten Winterhälfte, ein schwächeres im beginnenden Sommer. Während im Winter einige Keimblätter binnen drei Tagen grünen, ist im Sommer die Menge der explosiv grünenden Keimblätter geringer, doch sind dafür öfters in den Früchten bereits angekeimte Kerne oder Keimlinge anzutreffen. Besonders im Juli und August fand der Verfasser in den Früchten der vorjährigen Ernte oft Keimlinge, manchmal mehrere in einer einzigen Frucht (Abb. 4). Nach Umpflanzung in die Erde wachsen sie sofort und intensiv bis zum Herbst.

Abb. 4. Offene Frucht der Sorte Ontario nach zehnmonatiger Lagerung. Im Inneren der Frucht befinden sich die Keimlinge. Photo V. Kopal

Die zahlenmässige Analyse ergab, dass das mögliche Vorkommen der Bioteknose von der Dauer der Lagerung (Alter der Frucht) und vom physiologischen Zustand der Frucht während der Lagerung abhängt. Die Bioteknose ist streng an die Möglichkeit der vollkommenen Grünung der Keimblätter gebunden, hängt aber auch von der Sorte ab. Interaktionen zwischen Sorte und Lagerungsdauer oder zwischen Grünungsgrad der Keimblätter und Sorte sind nicht signifikant (Taf. 1, 2).

Der Grünungsgrad der Keimblätter ist abhängig von der Fähigkeit zur Ausbildung eines vollständigen Chlorophyllapparats, eine Erscheinung genotypen Charakters. Es ist daher der Schluss zulässig, dass die hohe Vitalität isolierter Keimlinge und die Möglichkeit einer Bioteknose beim Apfelbaum nicht nur eine Frage des physiologischen Luxurierens ist, sondern dass es sich um eine Erscheinung der physiologischen und auch der mit dem hybriden Charakter des Keimlings verbundenen Kompatibilität handelt.

Der Verfasser beobachtete die Bioteknose auch bei den Sorten Nathusius Taubenapfel, Jonathan und Wilhelmapfel. Ein systematisches Studium dürfte sie auch bei anderen Apfelsorten nachweisen.

Die hier beschriebene Methodik des Studiums der Vitalität isolierter Keimlinge erlaubt in einer verhältnismässig kurzen Zeit (7 Tage) die künstliche Auswahl des Materials für das weitere Studium der Sorte. Der Kenntnis der Vitalität der Keimlinge kommt bei der beabsichtigten Kreuzung von Sorten eine besondere Bedeutung zu.

Tab. 1. Untersuchung der Vitalität exstirpierter Keimlinge der Apfelsorten (Klarapfel K; Wealthy: W; Ontario: O) während der Lagerung (vom Oktober, X, bis März, III) nach der Keimblattsgrünung (y_i).

Grünungsgrad y_i	Sorte	Monate X	XI	XII	I	II	III	Summen
y_0:	K	0	0	0	0	1	1	2
	W	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0
Summe		0	0	0	0	1	1	2
y_1 :	K	0	0	0	0	1	0	1
	W	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0
Summe		0	0	0	0	1	0	1
y_2:	K	0	0	0	1	0	1	2
	W	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0
Summe		0	0	0	1	0	1	2
y_3:	K	1	1	0	0	0	0	2
	W	1	1	0	0	0	0	2
	O	1	0	1	0	0	0	2
Summe		3	2	1	0	0	0	6
y_4	K	2	2	0	0	2	2	8
	W	2	2	0	0	2	2	8
	O	2	2	2	0	0	0	6
Summe		6	6	2	0	4	4	22
y_5:	K	2	2	2	3	2	0	11
	W	2	2	2	2	2	2	12
	O	2	2	2	2	0	0	8
Summe		6	6	6	7	4	2	31
y_6:	K	2	2	3	3	0	0	10
	W	2	2	2	2	2	2	12
	O	2	2	2	3	3	2	14
Summe		6	6	7	8	5	4	36
y_7:	K	3	3	5	6	2	2	21
	W	2	2	2	2	2	2	12
	O	0	2	2	4	4	3	15
Summe		5	7	9	12	8	7	48

Fortsetzung der Tabelle 1.

Grünungsgrad y_i / Sorte		Monate X	XI	XII	I	II	III	Summen
x_8:	K	3	4	7	10	7	5	36
	W	0	3	3	4	3	3	16
	O	0	2	4	5	5	3	19
Summe		3	9	14	19	15	11	71
y_9:	K	5	7	15	20	13	8	68
	W	0	0	5	5	5	5	20
	O	0	3	6	23	16	4	52
Summe		5	10	26	48	34	17	140
y_{10}:	K	19	25	31	39	42	47	203
	W	0	0	34	42	38	35	149
	O	0	22	26	37	52	49	186
Summe		19	47	91	118	132	131	538
y_{0-10}:	K	37	46	63	82	70	66	364
	W	9	12	48	57	54	51	231
	O	7	35	45	74	80	61	302
Summe		53	93	156	213	204	178	897

Tab. 2. Ergebnisse der Streuungsanalyse.

Variabilität	Summe der Quadrate	Freiheitsgrad	Streuung	Testkriterium F	Kritische Beträge $F_{0\cdot05}$	Kritische Beträge $F_{0\cdot01}$	Signifikanz
Keimblattgrünung	13668	11 — 1 = 10	1366·8	176·62	1·92	2·51	sehr hoch
Alter gelagerter Früchte (Monate)	617	6 — 1 = 5	123·4	15·94	2·30	3·20	hoch
Sorte	134	3 — 1 = 2	67·0	8·66	3·09	4·82	hoch
Lagerungsalter (Monate) und Keimblattgrünung	3599	5 . 10 = 50	71·98	9·30	1·48	1·73	hoch
Monate und Sorte	60	5 . 2 = 10	6·00	0·78	1·92	2·51	nicht signifikant
Keimblattgrünung und Sorte	369	10 . 2 = 20	18·45	2·38	1·68	2·06	nicht signifikant
Residualvariabilität	774	5 . 10 . 2 = 100	7·74				
Gesamte Variabilität	19221	6 . 11 . 3 — 1 = 197					

Literatur

Audus, L. V.: Plant Growth Substances. London 1953.
Beneš, M., Likeš, J.: Faktorové experimenty v průmyslovém výzkumu (Faktorenexperimente in der Industrieforschung). Pokroky matem., fys. a astron., **2** : 18—30 und 156—166, 1957.
Bělochonov, I. V.: Ovocnářství. Praha 1953.
Crocker, F., Barton, L. V.: Physiology of Seeds. New York 1953.
Kobel, F.: Lehrbuch des Obstbaumes auf physiologischer Grundlage. Berlin 1954.
Mičurin, I. V.: Spisy. 1. Principy a metody práce (Schriften. 1. Prinzipien und Methoden der Arbeit). Praha 1953, S. 271.
Myslivec, V.: Statistické metody zemědělského a lesnického výzkumnictví (Statistische Methoden des land- und forstwissenschaftlichen Forschungswesens). — Praha 1957.
Němec, B.: Život rostlin. II. (Das Leben der Pflanzen.) Praha 1941.
Němec, B.: Pohyby a rozmnožování rostlin. Fysiologická anatomie rostlin (Bewegungen und Vermehrung der Pflanzen. Physiologische Anatomie der Pflanzen). Praha 1944.
Passecker, F.: „Lebendgebärende" Äpfel und Tomaten. Gartenzeitung, Organ d. österr. Gartenbaugesl. in Wien **13** : 6—7, 1937.
Petrů, E., Řetovský, R.: Rostlinné explantáty (Pflanzliche Explantate). Praha 1956.
Ulbrich, E.: Biologie der Früchte und Samen (Karpologie). Berlin 1928.
Velenovský, J.: Všeobecná botanika, Srovnávací morfologie. III. (Allgemeine Botanik. Vergleichende Morphologie. III.). Prag 1910.
Zelinková, M., Hořavka, F., Řetovský, R.: Klíčení jasanu ztepilého (Fraxinus excelsior L.) — (Die Keimung der Edelesche). Sborník ČAZ (Praha) **24** : 94—101, 1951.

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Биотекноз у яблони

РУДОЛЬФ РЖЕТОВСКЫ

Резюме

Автор часто находил летом в складах фруктов яблок сорта Ontario с сеянцами. Это явление, называемое биотекнозом, автор наблюдал также ранней весной на плодах сорта Wealthy. Поэтому он исследовал систематически скорость позеленения и рост эмбриональных корешков изолированных зародышей яблони сорта «Прозрачное желтое (летнего), Wealthy (осеннего) и Ontario (зимнего сорта) в течение хранения плодов в складе.

Экстирпированные зародыши выращивались на вате в чашках Петри в течение 7 дней в строго определенных условиях температуры и освещения. Результаты опытов были обработаны статистически путем анализа дисперсии. Скорость позеленения семядолей и интенсивность роста прорастающего корешка зависят от сорта и от времени хранения (возраста плода). Для каждого из изучаемых сортов существует важный период, когда изолированные из хранимых плодов зародыши отличаются повышенной жизнедеятельностью (необыкновенно быстро прорастают, зеленеют, и эмбриональный корешок быстро удлиняется). Зародыши с этими свойствами, как и сеянцы, найденные прямо в плодах, после пересадки в землю растут немедленно и интенсивно. Короткий период выращивания изолированных зародышей (7 дней) позволяет произвести отбор наиболее жизнеспособных зародышей.

Автор полагает, что наблюдавшиеся им явления могут быть использованы в плодоводстве.

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Gradients of Osmotic Pressure of Cell Sap in the Area of one Leaf Blade

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Souhrn

Nejen podle výšky inserce (zákon Zalenského), nýbrž také v rámci jedné listové čepele existují rozdíly v hydrataci. Byly zjištěny značné délkové, transversální i plošné gradienty osmotického tlaku buněčné šťávy (stanovení nepřímo indexem lomu podle empiricky zjištěné korelace mezi indexem lomu a osmotickým tlakem kryoskopicky změřeným [obr. 1]) v rozsahu jedné listové čepele cukrovky. Gradient base čepele-vrchol se pohyboval kolem +40 % osmotického tlaku buněčné šťávy na basi. Tento a ostatní gradienty ani jejich rozložení se zřetelně nezměnily ani při umělém dosycení listů vodou ani při vzniku nebo vyrovnání vodního deficitu aktuálními změnami vodní bilance. (Při těchto pokusech byla zachována polarita transpiračního proudu.) Tato zjištěná stabilita různého stupně hydratace jednotlivých částí dospělé čepele je důkazem toho, že toto rozrůznění je dáno předchozím ontogenickým vývojem listu jako polárního orgánu, a že se v dospělosti listu udržuje v podstatě bez ohledu na aktuální změny vodního provozu listu. Zjištěná heterogenita listu, pokud se týče uvedené veličiny, je výrazem fysiologické heterogenity listové čepele.

Summary

Within one leaf blade there are considerable differences in hydration of its parts. Marked longitudinal, transverse and area gradients of the osmotic pressure of cell sap were ascertained within the leaf blade of sugar beet. The osmotic pressure was determined indirectly by the refractive index according to the empirically established correlation refractive index: osmotic pressure (measured cryoscopically) cf. fig. 1. The gradient from the base of the blade to the tip ranged around plus 40% of the osmotic pressure at the base. This and other gradients mentioned were not noticably changed even when the leaves were artificially saturated with water or when water deficit arose or was adjusted by actual changes in the water balance. (In all these experiments the polarity of the transpiration stream was maintained.) The stability found in various degrees of hydration of individual parts of the adult leaf blade indicates that the variability is a result of the previous ontogenetical development of the leaf as a polar organ and of the fact that the adult leaf remains essentially unchanged by the actual changes in the water exchange. The heterogeneity found in the leaf with regard to the values here dealt with is an expression of the physiological heterogeneity of the leaf blade.

Introduction

Within the plant organism there exist progressive changes in the physiological (and as a consequence also in the morphological) characters of organs, their parts, tissues and cells, as a function of their relative positions (Prat, H. 1948 and 1951). These physiological and morphological gradients are primarily determined by the previous development of these parts. Many of them are

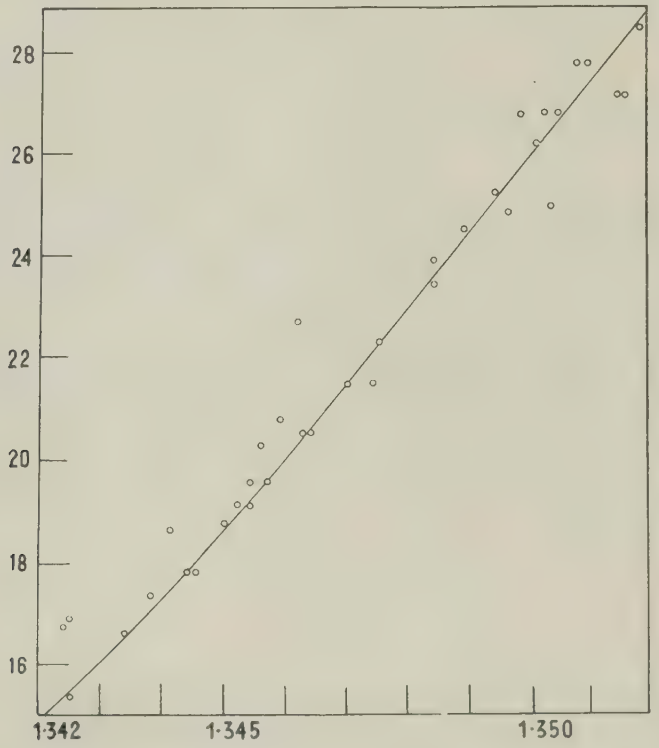

Fig. 1. Relation of osmotic pressure of cell sap (ordinate in atm.) of sugar beet leaves to their refractive index (abscissa) in the experimental period (October 1957).

closely and directly connected with the water relations and their polarity. The existence of axial gradients of the anatomical and physiological features of xeromorphism in leaves, according to the level of insertion is known (Zalensky's law). Recently their physiological investigation has been linked with the study of the physiology of xerophytes (recent work by Genkel' 1946, Simonis 1941 and 1952, Znamensky 1948, Maximov 1952, Stocker 1954, 1956, Killian and Lemée 1956, Farkas and Rajháthy 1955, Cetl 1957, and others).

In view of the polarity of leaves during ontogenesis the existence of physiological gradients within a single leaf blade may be anticipated. Heterogeneity within the leaf blade has not yet been investigated, although it is theoretically

probable (HUBER 1956). As an indicator of this anticipated heterogeneity, connected primarily with the character of water state and balance, the quantitative distribution of the hydration of leaf tissue within the leaf blade of sugar beet was examined. In seeking the cause of this heterogeneity the fundamental question was whether with varying water balance it is connected solely or primarily with the actual varying supply of water to different parts of the leaf blade, or whether there is a relatively stable heterogeneity already determined by the ontogenetical development of the leaf.

Methods

The correlation graph (fig. 1), drawn up *ad hoc* on the basis of 36 concurrent determinations of the refractive index (Abbe's universal refractometer) and the cryoscopic measurement of the osmotic pressure of cell sap from killed (100° C) leaf tissue of sugar beet, shows a practically linear relation between the two values within the limits of the experimental period (October). This relation can be regarded as reliable for practical application.*) This made it possible to make a sufficiently accurate estimate of osmotic presure from the refractive index, i. e. osmotic pressure could be measured in a manner which enabled the required large number of small samples of leaf tissue to be dealt with. In this way the osmotic pressure of cell sap from individual killed sectors of the leaf blade of sugar beet (*Beta vulgaris* ssp. *esculenta* [SALISB.] GÜRKE var. *altissima* ROESSIG) variety *Dobrovická A* was indirectly measured. For these experiments leaves of medium insertion level were gathered from the end of September to the end of October 1957. The osmotic value measured was regarded as a quantitative expression of the hydration of the tissue. Each half of the leaf blade was divided in each case into eleven sectors (fig. 2 and 3), the absolute size of which was determined by the absolute size of the leaf blade. This varied around an average of 27 × 18 cm. The values given were determined with different controlled states of water saturation and with different water balances in natural and artificial experimental conditions. The results recorded represent averages from a larger number of determinations (*n* about 20 to 30). A total of 1,300 samples was dealt within the period stated.

I wish to express my sincere thanks to Mrs. M. Krejcarová for her technical assistance.

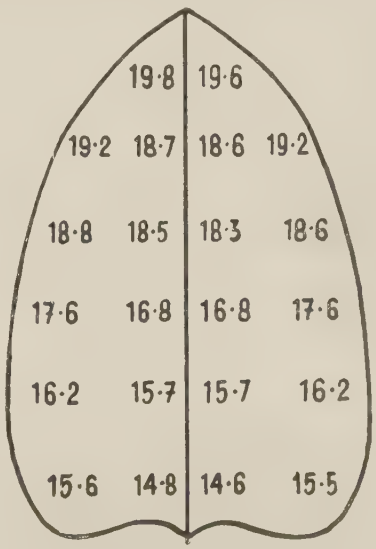

Fig. 2. Symmetrical distribution of osmotic pressure of cell sap (in atm.) over the area of sugar beet leaf blades (n = 16).

Results

With regard to the refractive index of cell sap, both halves of the blade of an

*) The existence of this practically linear relation of the values is limited to a quite definite developmental stage and to a definite part of the vegetative period. It is in fact only valid insofar as the relative quantitative composition of the osmotically active substances dissolved in the cell sap does not undergo any marked change (SLAVÍK 1959). The relation illustrated in fig. 1 is not quite linear. Under the simplifying assumption of linearity its correlation coefficient is $r = +0{\cdot}98$, regression coefficient $b_y = 1{\cdot}37$ (atm. to thousandth units of the refractive index)

adult sugar beet leaf were mutually symmetrical, both relatively and absolutely (fig. 2). Corresponding values differed from each other on an average by less than 1%, maximally by 1·5%. This result is especially important for observation of the relative changes in osmotic pressure from corresponding places, when the water balance is active or passive and of varying intensity.

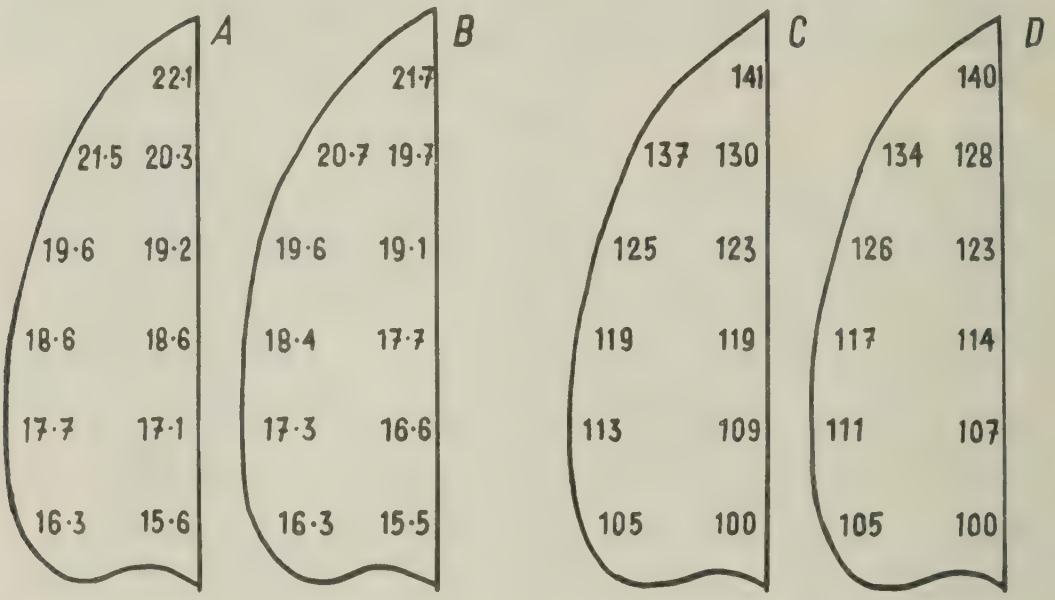

Fig. 3. Distribution of osmotic pressure of cell sap on half the surface of the leaf blade of sugar beet in two sets of experiments (A and B in atmospheres, C and D in percentage of osmotic pressure at the base of the blade; A and C : n = 36, B and D : n = 31).

These changes have been studied in another paper. Here we were concerned only with establishing the changes in gradient within the leaf blade, i. e. with the comparison of values within the same half of the leaf blade. The symmetry of the two halves of the blade gives a guarantee that the gradients in the two halves behave similarly under similar conditions.

The distribution of the average osmotic pressure of cell sap within one half of the sugar beet leaf under natural field conditions in the morning (8 a. m.) state of water saturation (resaturation at night) for two sets of experiments (n = 36 and 31) provides a basic survey (fig. 3). The graphic illustration of the axial gradient base of the blade tip along the main ribs (figs. 4, continuous lines) as a percentage of the osmotic pressure at the base shows that osmotic pressure increase is roughly linear according to position in relation to the longitudinal axis of the blade, so that the gradient remains essentially the same along the whole length of the blade. In this and other similar graphs it is possible to observe a weak, but in almost all cases clear reduction in the longitudinal osmotic gradient in the middle of the upper half of the leaf blade (between points 3 and 4).

A similar distribution of osmotic pressure of cell sap was ascertained in leaves which had been artificially saturated with water (20 hours with petioles in water in an atmosphere of 100% humidity and in the dark). The longitudinal gradient was the same (fig. 4, dashed lines) only the gradient at the tip being smaller.

Cut leaves (without petioles) allowed to wilt freely also maintain the same type of gradient (fig. 5). In comparison with the control (the second half of the leaf saturated at the start of wilting) this gradient does indeed show a slight change, but no relation was found between these changes and the magnitude or intensity of the water deficit of the leaf due to wilting.

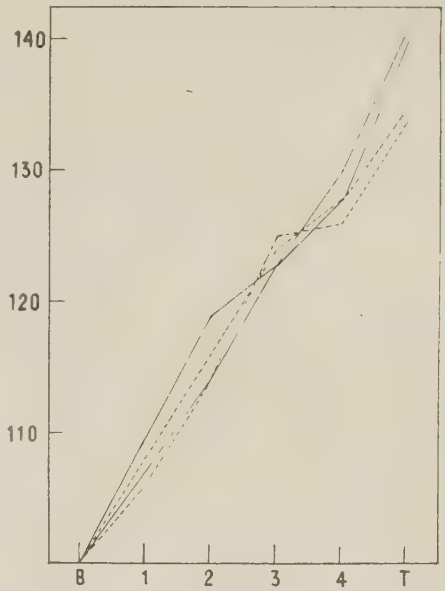

Fig. 4. Relation of the osmotic pressure of cell sap (ordinate in per cent of osmotic pressure at the base of the blade) to the position along the longitudinal axis of the blade (abscissa: B base of blade, T tip). Graphic representation of axial gradient.

Continuous lines: leaves picked in the morning after normal overnight saturation with water (n = 31 and 36). Dashed lines: artificial saturation of leaves (n = 16 and 16).

In another set of experiments the influence of a passive water balance (i. e. "wilting"), without interruption of the supply of water to the blade through the petiole, was studied. Water deficit in leaves with their petioles in water was caused by artificial experimental conditions (ventilation, radiation with an infra-red lamp). This set of experiments was particularly designed to show whether the area and longitudinal gradients of osmotic pressure of cell sap are noticeably changed by the assumed variation of water supply to the different parts, i. e. by varying water balance. Not even here, however, did the distribution and gradients change to any noticeable extent. Changes observed again concerned only the gradients at the tip (fig. 6) and their relation to the absolute magnitude of the water deficit and to the rate of its increase are clear from the values given in the description. It can be seen from the results that very small and medium deficits and their rate of increase did not change the gradient as compared with the control, while deficits of about 5% and large deficits (over 10%) reduced the tip gradient. But not even these changes disturbed the general course of the gradients.

Neither did an active water balance change this situation. With leaf blades in which the water deficit was artificially produced and which were then saturated with the petioles in water in an atmosphere saturated with water vapour and in the dark, the area distribution of the values under observation was also little changed as compared with the control (wilted half of leaf blade before resaturation) (fig. 7).

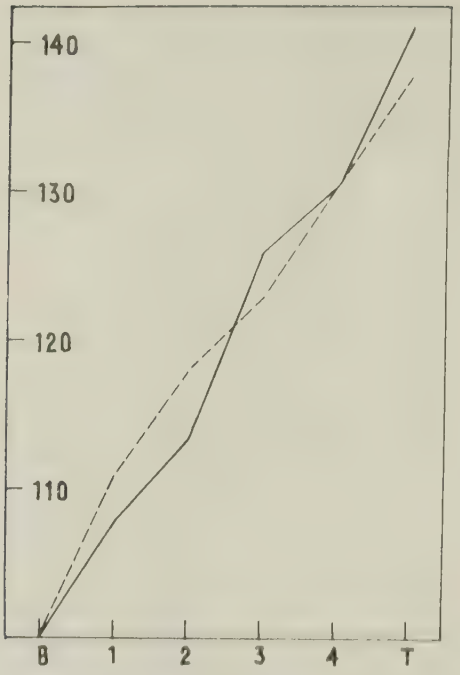

Fig. 5. Similar representation of the axial gradient as fig. 4. Continous line: wilting leaf blade without water supply through petiole. Dashed line: axial gradient of the second half of the blade before wilting (n = 26).

In general it can be said that neither saturation of the leaf with water, nor passive, nor active water balance (the occurrence or adjustment of total water deficit), with water supplied through the petiole or not, changed the relative distribution of osmotic pressure of cell sap over the area of the leaf blade, because all changes in these values took place in parallel over the whole area.

Discussion

Considerable differences were ascertained in the hydration of different parts of the sugar-beet leaf blade and these corresponded to the natural gradient along the main axis and transversely (from edge to centre). Both halves of the blade behaved quite symmetrically. The positive gradient of the osmotic presure of cell sap from the base of the leaf blade to the tip and from the mid-rib to the edge is an expression of a decreasing degree of

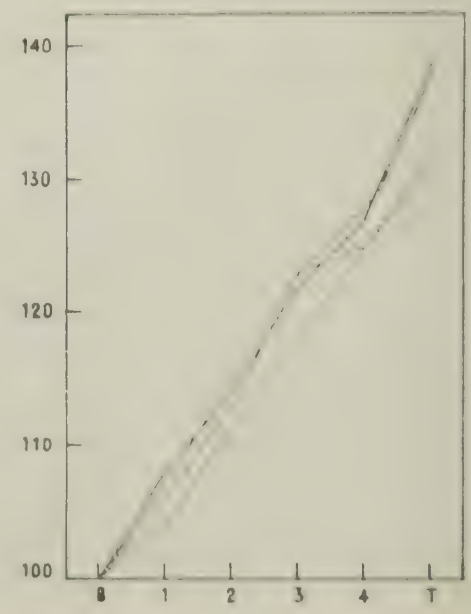

Fig. 6. Similar representation of axial gradient as fig. 4 and 5; passive water balance with water supply through petiole.

——— water deficit 0 to 2%, average 1%, average rate of increase of 0·6% per hour;

– – – – water deficit 2 to 6%, average 5·1%, average rate of increase 2·2% per hour;

— · — · — water deficit 6 to 10%, average 8·3%, average rate of increase 8·8% per hour;

– – – – water deficit > 10%, average 14·4%, average rate of increase 15% per hour.

hydration of the leaf tissues. The gradient from the base to tip in full grown leaves of sugar beet in the autumn was around 40% of the osmotic pressure at the base.

These gradients were not changed to any extent with actual changes in the state of water saturation as a result of different water balances. This indicates that these gradients (hydration gradients) are relatively permanent characters ensuing from the polarity of growth and the ontogenesis of the leaf, because when the leaf blade is mature they are maintained essentially unchanged without regard to actual changes in the water balance. It follows logically that this more or less permanent hydration degree of various parts of the area of the leaf blade undoubtedly arose as a result of permanently differing water balances of those parts in the early stages of development and growth of the leaf blade. It is, however, very probably connected both as cause and effect with differing intensities of other physiological processes. It may be expected that similar differences such as were ascertained for leaves at different levels of insertion and of different degrees of xeromorphism exist analogically within a single leaf blade. The magnitude of the gradients observed is considerable and corresponds to similar gradients according to height of insertion. At the same time it provides the opportunity of following such changes consecutively on the identical material.

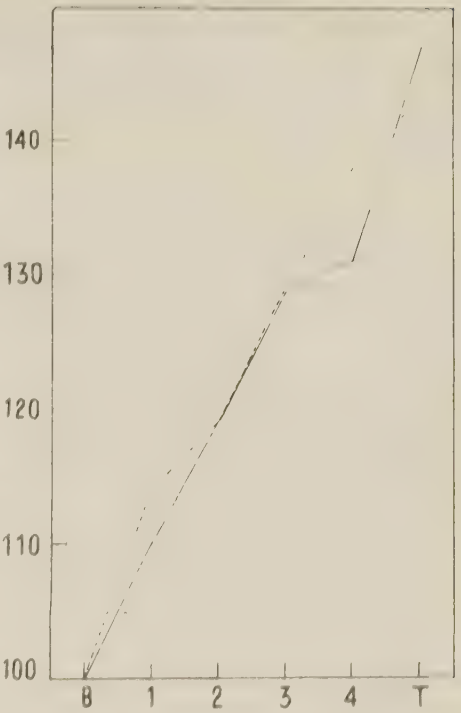

Fig. 7. Similar representation of axidal gradient as in the previous graphs. Saturation of wilted leaf blades. Continuous line: originally wilted, now saturated halves of leaf blades. Dashed line: second halves of leaf blades with original deficits (average 8·5%).

The significance of these gradients of osmotic pressure of the cell sap of leaf tissues for processes in the leaf itself can lie both in influencing the transpiration stream (Huber 1956, Levitt 1956) and in influencing the intensity of the transpiration of different parts of the leaf blade. As far as the first case is concerned it must be remembered that the factor which determines the osmotic absorption of water by the tissues is not the osmotic pressure of the cell sap (which is an indicator of hydration), but DPD (diffusion pressure deficit), i. e. the so-called "suction power" of the tissue (Huber 1956). The gradients of osmotic pressure of cell sap determined need not, therefore, quantitatively correspond to the DPD gradient of the tissue. Qualitatively, however this gradient of osmotic pressure of cell sap is a proof of the existence of analogical DPD gradients determining the osmotic absorption of water in the course of the transpiration stream. As far as the influence on transpir-

ation intensity is concerned, it has been theoretically established that only after the reduction of turgor to nought does increased osmotic pressure of cell sap start to act as a factor in reducing transpiration, and that this reduction is under normal conditions comparatively small (Renner 1915, Stalfelt 1956).

References

Cetl, I.: Odolnost polních plodin vůči suchu a možnosti jejího zvýšení. (The drought resistance of field crops and the possibility of increasing it.) — Rozpravy ČSAV **67** (8) : 1—107, 1957.

Farkas, G. L., Rajháthy, T.: Untersuchungen über die xeromorphischen Gradienten einiger Kulturpflanzen. — Planta **45** : 535—548, 1955.

Genkel', P. A.: Ustoychivost rasteny k zasukhe i puti eyo povysheniya. (The drought resistance of plants and means of increasing it.) — Moskva 1946.

Huber, B.: Die Wasserleitung. Die Gefässleitung. — Hb. d. Pflanzenphys. Bd. III, 541—582. l. cit. 575—578, Berlin—Göttingen—Heidelberg, 1956.

Killian, Ch., Lemée, G.: Les Xérophytes: leur économie d'eau. Hb. d. Pflanzenphys. Bd. III. 787—824, Berlin—Göttingen—Heidelberg, 1956.

Levitt, J.: The physical nature of transpirational pull. — Plant Physiol. **31** : 248—251, 1956.

Maximov, N. A.: Izbrannye raboty po zasukhoustoychivosti i zimostoykosti rasteny. Tom I. Vodny rezhim i zasukhoustoychivost rasteny. (Selected papers on drought and cold resistance of plants. Vol. I. Water relations and drought resistance.) — Moskva 1952.

Prat, H.: Histophysiological gradients and plant organogenesis. — I. Bot. Rev. **14** : 603—643. 1948, II. Bot. Rev. **17** : 693—746, 1951.

Renner, O.: Theoretisches und Experimentelles zur Kohäsionstheorie der Wasserbewegung. — Jb. wiss. Bot. **56** : 611—667, 1915.

Simonis, W.: Über den Einfluss des Bodenwassergehaltes auf die Kohlensäureassimilation und den Ertrag bei Einjährigen. — Ber. Dtsch. bot. Ges., **56** : 52—68, 1941.

Simonis, W.: Untersuchungen zum Dürreeffekt. I. Morphologische Struktur, Wasserhaushalt, Atmung und Photosynthese feucht- und trockengezogener Pflanzen. Planta **40** : 313—332. 1952.

Slavík, B.: The relation of the refractive index of plant cell sap to its osmotic pressure. — Biol. Plant. **1** : 48—53, 1959.

Stalfelt, M. G.: Die stomatäre Transpiration und die Physiologie der Spaltöffnungen. Hb. d. Pflanzenphys. Bd. III. 351—426, Berlin—Göttingen—Heidelberg, 1956.

Stocker, O.: Die Trockenresistenz der Pflanzen. 8ème Congr. Int. de Bot., Paris, 223—232, 1954.

Stocker, O.: Die Dürrenresistenz. Hb. d. Pflanzenphys. Bd. III : 696—741. Berlin—Göttingen—Heidelberg, 1956.

Znamensky, I. E.: Fiziologicheskaya i biochimicheskaya kharakteristika xerofitov. (Physiological and biochemical characteristics of xerophytes.) Eksper. Bot. (AN SSSR) **6** : 93—146, 1948.

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Градиенты осмотического давления клеточного сока в пределах пластинки листа

БОГДАН СЛАВИК

Резюме

Существуют значительные различия в степени гидратации не только в зависимости от яруса (закон Заленского), но и в пределах пластинки одного листа. Были найдены значительные продольные, поперечные и плоскостные градиенты осмотического давления клеточного сока [определявшиеся косвенно, в зависимости от индекса преломления, по эмпирически установленной корреляции между индексом преломления и криоскопически определяемым осмотическим давлением (рис. 1.)] в пределах пластинки одного листа сахарной свеклы. Градиент основание пластинки — верхушка пластинки колебался около +40% осмотического давления клеточного сока в основании листа. Ни этот, ни остальные градиенты, ни их распределение не менялись сколько-нибудь заметно даже при искусственном дополнении насыщения листьев водой или при возникновении или же устранении водного дефицита в результате актуальных изменений водного баланса. (При этих опытах оставалась неизменной полярность транспирационного тока.) Установленная нами устойчивость различных степеней гидратации отдельных частей взрослого листа является доказательством того, что эти различия обусловлены предшествующим онтогенетическим развитием листа как полярного органа и что в стадии взрослого листа она сохраняется, по существу, независимо от актуальных изменений водного баланса листа. Указанная гетерогенность листа является выражением физиологической гетерогенности пластинки листа. Эта гетерогенность касается, несомнено, не только водного режима листа, но и остальных его физиологических свойств.

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The Relation of the Refractive Index of Plant Cell Sap to its Osmotic Pressure

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Souhrn

Byla provedena paralelní měření osmotického tlaku (stanoven kryoskopickou metodou) a indexu lomu buněčné šťávy u 246 vzorků ze sedmi druhů rostlin (bylin i dřevin). Zároveň byly zjištěny obě hodnoty a jejich závislost u vodných roztoků některých cukrů, organických kyselin a anorganických solí. Závislost obou hodnot u těchto modelových roztoků je u různých látek různá. Kvantitativní složení osmoticky působících složek buněčné šťávy se mění v průběhu ontogenese a tím se mění i závislost indexu lomu a osmotického tlaku. Tímto srovnáním bylo ukázáno, že spolehlivá a velmi dobře prakticky využitelná více méně lineární závislost obou veličin platí vždy pro omezený časový úsek ontogenese pokusné rostliny. Stanovení osmotického tlaku buněčné šťávy podle jejího indexu lomu, jehož hlavními přednostmi je rychlost a malý potřebný objem šťávy (0,02 ml), je spolehlivé tehdy, sestavíme-li pro pokusnou rostlinu a omezený úsek ontogenese empiricky *ad hoc* příslušný korelační graf.

Summary

Parallel measurements of the osmotic pressure (cryoscopic method) and the refractive index of cell sap were carried out on 246 samples from seven plant species. At the same time both values were ascertained as well as their relation for aqueous solutions of some sugars, organic acids and inorganic salts. The relations found with these model solutions are different for different substances. The quantitative composition of osmotically active components of cell sap changes in the course of ontogenesis and as a result the relation of refractive index and osmotic pressure also changes. By this comparison it has been shown that a reliable and more or less linear relation of the two values, which is advantageous for practical application, is only valid for a limited period in the ontogenesis of the experimental plant. The determination of the osmotic pressure of cell sap from the refractive index, the main advantage of which is its quickness and the small volume of sap required (0·02 ml.), is reliable if we construct empirically *ad hoc* the appropriate correlation graph for the experimental plant for a limited ontogenetic period.

Introduction

The osmotic pressure of cell sap is an important indicator of the hydration of tissues. From the physical point of view it definitely determines the deficit of the diffusion pressure of water in this solution. The exact method of determination by cryoscopy requires an excessively large quantity of sap (about 1 ml.) for the normal procedure, its submicromodifications are technically exacting (e. g. Mosebach 1940). Osmotic pressure is given by the number of particles (ions, molecules) of dissolved substance and in contradistinction to concentration (by weight), depends to a great extent on their dissociation. The concentration of a solution of constant composition can be determined (within certain limits of dilution) on the basis of the refractive index of the solution. Since the determination of the refractive index can be carried out very quickly and sufficiently accurately even with a small amount of solution (0·02 ml.), it would be very advantageous if we could use refractometry for the determination, or only for the estimation, of the osmotic pressure of cell sap. The refractive index has already been used in this way (Lobov 1949) under the simplified and incorrect assumption that the osmotic pressure of cell sap is primarily determined in plants by the concentration of monosaccharides. The analysis of cell sap, however, shows (Steiner 1933, and 1939, Knodel 1938, Slavík 1955, Pisek 1955) that the main components to act osmotically are first inorganic salts, then sugars, organic acids and their salts.

The use of the refractive index of cell sap for the direct determination of its osmotic pressure without any reservations is made difficult by the following facts: 1. The relation of the refractive index to concentration is different for different substances. 2. The relation of osmotic pressure to concentration depends on the dissociation of substances, which in its turn depends not only on the molecular concentration, but also in mixtures on the concentration of individual ions and of other ions with which association or some other chemical reaction may take place. Therefore a change in the composition or quantity of the substances dissolved in the cell sap changes the refractive index and osmotic pressure in various ways.

The aim of our measurements was to find out to what extent and with what limitations it would, in spite of these facts, be possible to use the refractive index in estimating the osmotic pressure of cell sap.

Methods

A total of 246 concurrent cryoscopic determinations of osmotic pressure was carried out (simplified method, Slavík 1952) and the refractive index of cell sap (at 20° C), which had been expressed from leaves killed at 100° C was determined. These leaves were taken from 7 species of plants (herbaceous and woody) at different stages of ontogenesis of the leaves throughout the whole vegation period of 1956, some measurements being made in 1957. The experimental plants were sugar beet, *Beta vulgaris*, subsp. *esculenta* (Salisb.) Guerke, var. *altissima* Roessig (Dobrovická A variety); *Fagus silvatica* L., *Quercus petraea* Liebl, *Carpinus betulus* L., *Alnus glutinosa* Gaertn., *Acer pseudoplatanus* L., *Salix capraea* L. Both these values were determined in a similar way for aqueous solutions of some chemically pure (puriss. and p. a.) sugars, organic acids and inorganic salts (glucose, sucrose, oxalic acid, malic acid, KCl, KNO_3, Na_2SO_4) and their mixtures.

I wish to express my sincere thanks to Mrs. M. Krejcarová for her technical help.

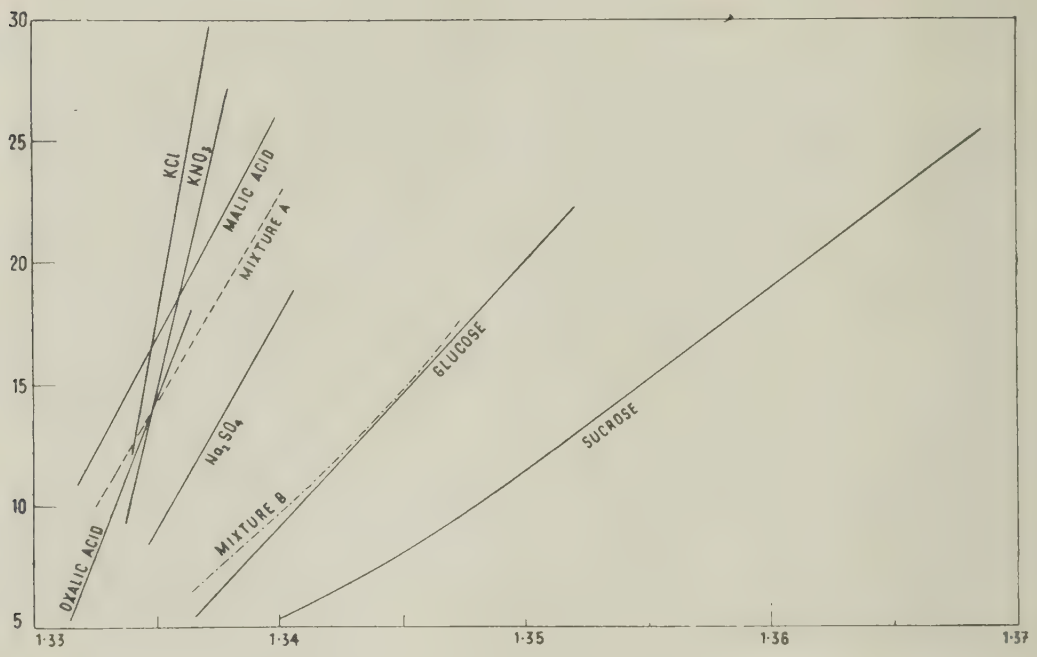

Fig. 1. Relation of the osmotic pressure of a solution (ordinate, in atm.) to the refractive index (abscissa) for aqueous solutions of pure osmotically important normal components of cell sap of plants (continuous line) and their mixtures; dotted: mixture A: 0·35 M sucrose +0·8 M oxalic acid +0·67 M KCl 1 : 1 : 1; dot-dash: mixture B: 0·4 M sucrose + 0·8 M oxalic acid + 0·4 M KCl 1 : 1 : 1. The lines are somewhat schematic; the relation is not in fact exactly linear.

Results and Discussion

From both series of findings correlation graphs between the refractive index and osmotic pressure were drawn up. In solutions of individual chemically pure components the varying relation of the refractive index and osmotic pressure are clearly seen. With inorganic salts, when the osmotic pressure rises steeply the refractive index changes very little, while, for example, with solutions of sucrose even small changes in osmotic pressure give distinct changes in the refractive index (fig. 1). In particular, the comparison of the two mixtures (A, B, fig. 1) shows very clearly how the individual components and their concentrations have a varying effect on the resultant relation.

In view of the fact that the qualitative and quantative composition of the cell sap of a plant or of its parts changes considerably during ontogenesis, it could be expected that the relation between the two values would be different at different stages of the vegetation period. Examples shown in graphs 2 and 3 demonstrate this. In the spring months there is clearly a preponderance of inorganic salts among the osmotically active components of cell sap in young leaves. This is apparent from the fact that the line of the relation of osmotic pressure to the refractive index is steep, while later, with an increase in gly-

cides, it rises more gradually. This was in general repeated with all species investigated. Naturally the line is not even, because changes in the ecological factors acting on the plant during the vegetation period influence the production of metabolites. Nevertheless, we can see that within a limited period of time

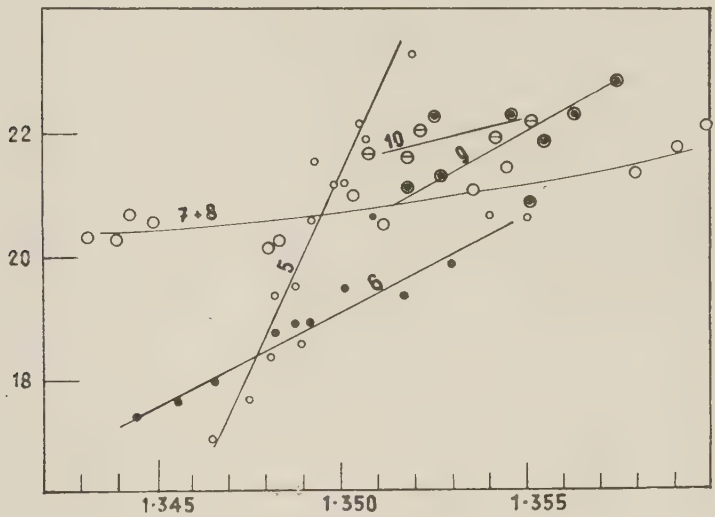

Fig. 2.

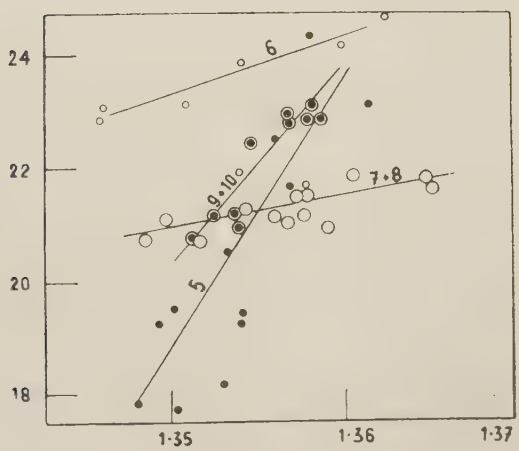

Fig. 3.

Fig. 2 and 3. Changes in the relation of osmotic pressure (ordinate in atm.) to the refractive index (abscissa) of cell sap from plants during vegetation period. The Arabic numerals by the lines representing a rough estimate of average correlation indicate months. Fig. 2: leaves of *Salix caprea* L., fig. 3: leaves of *Carpinus betulus* L.

there is a really good and applicable linear relation between the refractive index of cell sap and its osmotic pressure. Fig. 4 reproduces the relation of the two values for the cell sap of sugar beet leaves (of corresponding developmental age) using samples taken within about the period of a fortnight. Their correlation coefficients, calculated on the simplifying assumption of linear relation, are all higher than +0·98.

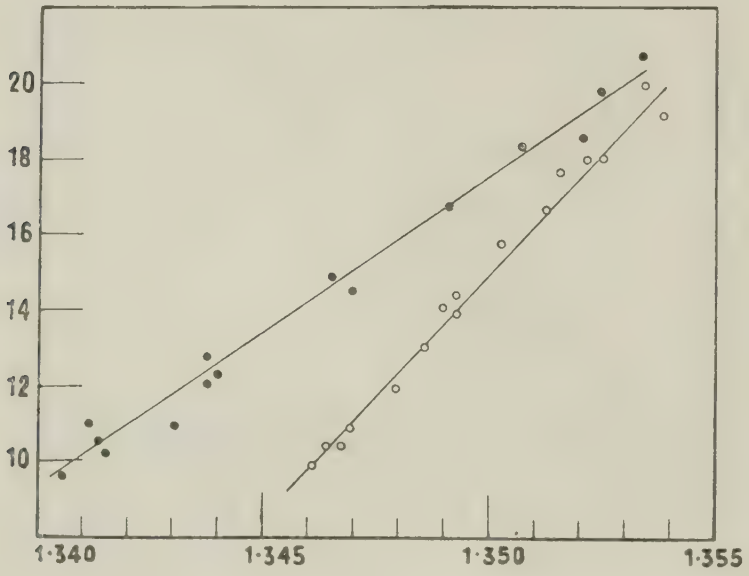

Fig. 4. Relation of osmotic pressure (ordinate in atm.) to the refractive index (abscissa) of cell sap from leaves of sugar beet (dots) from samples taken within a fortnight in the first half of August 1957 and from oak leaves (*Quercus petraea* Liebl) (circles) in the same period.

A certain applicable relation between the refractive index and osmotic pressure of cell sap exists in all cases only within a limited period of the plant's development, because this relation is influence by the changes in the chemical composition of the cell sap during the ontogenesis of a plant or its organs. It is, therefore, possible to use the refractive index in order to obtain a more or less satisfactory and sufficiently accurate estimate of the osmotic pressure of cell sap only by using a correlation graph, which must be drawn up by comparing the refractive index and the osmotic pressure determined cryoscopically in each case *ad hoc* for the given plant and organ and for a definite time period. The shorter the period and the simpler the material and the ecological conditions, the more reliable is the estimate. For similar estimates it is not possible for example to use the relation between the refractive index and osmotic pressure of solutions of individual components of cell sap, e. g. monosaccharides or sucrose, with any degree of reliability.

Under the conditions described and with the reservations mentioned it is possible and advantageous to use the refractive index of cell sap as a quantative indicator of its osmotic pressure in all cases where it is necessary to work with small quantities of sap (0·02 ml.) and at the same time to investigate a large number of samples.

References

Knodel, H.: Eine Methode zur Bestimmung der stofflichen Grundlagen des osmotischen Wertes von Pflanzensäften. — Planta **28** : 704—715, 1938.

Lobov, M. F.: K voprosu o sposobakh opredeleniya potrebnosti rasteny v vode pri polivakh. (On the determination of water consumption by plants during watering.) — Dokl. AN SSSR **66** : 277—280, 1949.

Mosebach, G.: Ein Mikroverfahren zu kryoskopischen Untersuchungen saftreicher Gewebe. — Ber. Dtsch. bot. Ges. **58** : 29—40, 1940.

Pisek, A.: Chemie des Zellsaftes. Handbuch der Pflanzenphysiologie Bd. I. S. 614—626. Springer-Verlag, Berlin—Göttingen—Heidelberg, 1955.

Slavík, B.: Osmotické hodnoty dřevin jako indikátor vhodnosti pro stanoviště. (Osmotic values in woody plants as an indicator of their suitability for a habitat.) — Čs. biologie (Praha) **1** : 125—133, 1952.

Slavík, B.: K dynamice vodního deficitu rostlin. (On the dynamics of water deficit in plants.) — Preslia **27** : 124—153, 1955.

Steiner, M.: Zum Chemismus der osmotischen Jahresschwankungen einiger immergrüner Hochgewächse. — Jb. f. wiss. Bot. **78** : 564—662, 1933.

Steiner, M.: Die Zusammensetzung des Zellsaftes bei höheren Pflanzen. — Erg. d. Biol. **17** : 151—254, 1939.

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Зависимость индекса преломления клеточного сока растений от его осмотического давления

БОГДАН СЛАВИК

Резюме

Были произведены параллельные измерения осмотического давления (с помощью криоскопического метода) и индекса преломления клеточного сока у 246 образцов 7 видов растений (травянистих и древесных пород). Одновременно определялись оба показателя и их зависимость у водных растворов некоторых сахаров, органических кислот и неорганических солей — в качестве модели. Зависимость обоих показателей в этих растворах не одинакова для различных веществ. Количественный состав осмотически действующих компонентов клеточного сока меняется в течение онтогенеза, и в связи с этим меняется и зависимость индекса преломления и осмотического давления. Это сравнение показывает, что надежная и весьма удобная для практического использования более или менее линейная зависимость обоих показателей остается в силе всегда лишь для ограниченного во времени отрезка онтогенеза подопытного растения. Определение осмотического давления клеточного сока по его индексу преломления, главным удобством которого является быстрота и потребность в небольших количествах сока (0,02 мл), надежно в тех случаях, если для подопытного растения и определенного отрезка времени можно эмпирически ad hoc составить соответственный корреляционный график.

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A Study of Metabolism of Exogenous Tryptophane and β-Indoleacetic Acid in Extirpated Wheat Embryos

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Souhrn

Pro objasnění látkových přeměn tryptofanu a kyseliny β-indolyloctové byly přežívajícím embryím pšenice v Knopově roztoku přidávány buď tryptofan nebo kyselina β-indolyloctová v koncentraci $3 . 10^{-2}$ M. Po 24 hodinách byly z embryí rychle připraveny alkoholické výluhy, které byly chromatograficky nebo elektroforeticky analysovány. Kromě chromatografických soustav a detekčních činidel, obvyklých při analyse indolových sloučenin, byly použity některé méně známé systémy, které se osvědčily při analyse nově objeveného indolového derivátu rostlin čeledi *Brassicaceae* — askorbigenu. Jsou to soustavy octan butylnatý nasycený vodou, tetrachlormetan—kyselina octová (100 : 2), formaldehydové detekční činidlo (40% formaldehyd—konc. HCl—voda 1 : 1 : 2). Papírová elektroforesa byla prováděna ve M/15 fosfátovém pufru pH 6.6 při 300 V po dobu 4 až 5 hodin. Hlavní pozornost byla věnována důkazu hydrofilních komplexů dodávaných látek. V embryích přežívajících v roztocích kyseliny β-indolyloctové byla kromě jiných indolových derivátů zjištěna přítomnost kyseliny β-indolylacetylasparagové. Její identita byla prokázána podle R_F v 5 různých soustavách barevnými reakcemi a chromatografickým důkazem jejich složek po alkalické hydrolyse (6 N $Ba(OH)_2$ po 30 min. při 100° C). V embryích přežívajících v tryptofanu byla zjištěna látka obdobných chromatografických vlastností jako kyselina indolylacetylasparagová, která pak však po hydrolyse poskytovala tryptofan. Předpokládá se, že jde o malonyltryptofan. Kromě toho byl v jednom pokusu pozorován vznik látky chromatograficky obdobné askorbigenu. Uvedená práce potvrzuje odlišnými prostředky a na jiném biologickém materiálu současné výsledky Andreaeho a Gooda.

Summary

The metabolism of exogenous tryptophane ($3 . 10^{-2}$M) and β-indoleacetic acid ($3 . 10^{-2}$M) in extirpated wheat embryos was studied chromatographically and electrophoretically.

It was established that the external application of β-indoleacetic acid in wheat embryos leads to the formation of its complex, indoleacetylaspartic acid. Chromatographical demonstration of the components of the complex was made following alkaline hydrolysis.

A complex compound also arises from exogenous tryptophane, which again frees tryptophane following alkaline hydrolysis. The assumption has been put forward that this is malonyltryptophane, reported by Andreae

and Good (DANNENBURG and LIVERMAN 1957). The isolated finding of a further substance may be mentioned, which possesses similar chromatographic properties to those of the indole complex—ascorbigen. For reasons so far unknown, this substance has not been isolated again in wheat plants.

Introduction

Many authors (SKOOG 1937, WILDMAN, FERRI and BONNER 1947, WILDMAN and MUIR 1949, GORDON and NIEVA 1949, ABRAMS 1953, HEMBERG 1955, PILET 1956) have found that tryptophane is often partially metabolized to growth substances in plant tissues. The metabolic processes involved in this conversion have not yet been elucidated and have formed the subject of many studies (LARSEN 1951, GORDON 1954). At the end of last year DANNENBBURG and LIVERMAN (1957) published a detailed study of the products of metabolism of tryptophane-2-C^{14} in tissue slices of the water melon and oat coleoptiles.

It can be assumed that, following the entry of indoleacetic acid into plant tissue, part of it will be decomposed by oxidase (TANG and BONNER 1947, MACLACHLAN and WAYGOOD 1956, RAY 1956). A further part of the heteroauxin is bound to protein (WILDMAN and GORDON 1942, WILDMAN and BONNER 1947, SIEGEL and GALSTON 1953). More recent work by Andreae et al. has shown that exogenous indoleacetic acid forms a complex compound, indoleacetylaspartic acid (ANDREAE and GOOD 1955, GOOD, ANDREAE and VAN YSSELSTEIN 1956, ANDREAE and VAN YSSELSTEIN 1956).

In the present study, we attempted to verify current knowledge of the metabolism of exogenous tryptophane and indoleacetic acid in wheat seedlings, using a somewhat different method. We were particulary interested in evidence of hydrophilic complexes of indole in the plant tissues.

Methods

Cultivation of plants: Wheat grains (Chlumecká 12, 1956 harvest) were disinfected for a quarter of an hour in 96% ethanol and rinsed several times in boiled distilled water. The grains were germinated in Petri dishes on rolls of filter paper moistened with Knop's solution—Zázvorka's modification (PETRŮ and ŘETOVSKÝ 1956). The Petri dishes were placed in a glass chamber and exposed to the normal alternation of day and night. The average temperature was 25° C. The grains germinated on the second day. When the average length of the coleoptiles was 5 mm. the embryos were extirpated.

Survival of the embryos in indole solutions: The extirpated embryos were kept in Knop's solution with $3 \cdot 10^{-2}$M tryptophane or $3 \cdot 10^{-2}$M β-indoleacetic acid. For 200 embryos 20 ml. of nutrient solution was used. In the control experiment the embryos were placed in Knop's solution. The Petri dishes were returned to the glass chambers. After a further 24 hours the embryos were removed from the solution and prepared for examination.

Preparation of extracts: The embryos were thoroughly rinsed with distilled water and dried of external water. They were then weighed and ground in a mortar with a small amount of silica sand. In the majority of experiments an alcohol extract was prepared (VALENTA, KUTÁČEK and ICHA 1956) — one part by weight of the plant material was made up to a total volume of three parts with 96% ethanol. The suspension was left in a refrigerator at a temperature of about +4° C for two hours before centrifuging. In some experiments sap pressed from the plants was shaken up with twice the volume of ethyl acetate.

Chromatographic separation of indoles and detection of chromatograms. Chromatography was carried out in a descending, or exceptionally in an ascending arrangement in the following systems

S_1 — butyl acetate saturated with water
S_2 — n-butanol—glacial acetic acid—water (4 : 1 : 5)
S_3 — carbon tetrachloride—glacial acetic acid (100 : 2)
S_4 — isopropanol—ammonia (d = 0·8951)—water (10 : 1 : 1)
S_5 — isopropanol—8N—NH_3 (8 : 2)
S_6 — isopropanol containing 1·25% acetic acid—20% aqueous solution of ammonium acetate (8 : 2)

Detection was carried out with a) a formaldehyde reagent (1 volume 40% formaldehyde mixed with 1 volume of concentrated hydrochloric acid and 2 volumes of water). Chromatograms were dried at 70° C. With this reagent only derivatives of indole carrying a substituent with a methylene group in the α-position to the indole ring in position 3 of the indole nucleus give a positive reaction, producing spots with yellow or orange fluorescence in ultraviolet light; b) Salkowski's reagent (50 volumes 5% perchloric acid mixed with 1 volume 0·05 M—$FeCl_3$; c) Ehrlich's reagent (2% solution of p-dimethylaminobenzaldehyde in a mixture of 20 ml. concentrated hydrochloric acid and 80 ml. 96% ethanol); d) 1% solution of cinnamic aldehyde in methanol; e) 0·1% solution of ninhydrin in n-butanol; f) 0·05% solution of Bromocresol Green in 96% ethanol; the pH is adjusted to 7.

Paper electrophoresis of the indole derivatives was carried out by the method of Ullman and Liebl (private communication) in M/15 phosphate buffer of pH 6·6 at 300 V for a period of 4 to 5 hours.

Alkaline hydrolysis of the complexes was carried out in 6N-$Ba(OH)_2$ on a boiling water bath for 30 min. (Andreae and Good 1955).

Results

A. Experiments with β-indoleacetic acid.

In alcohol extracts of embryos kept in $3 \cdot 10^{-2}$M solutions of β-indoleacetic acid the following spots were found on chromatograms obtained in system S_1 and detected with the formaldehyde reagent:

a) β-indoleacetic acid, R_F 0·86 (spot I).
b) a spot of R_F 0·71 showing brownish-yellow fluorescence (spot II).
c) a spot of R_F 0·05 showing orange-pink fluorescence (spot III).

On some chromatograms a yellow fluorescence, which we consider be due to tryptophane was present at the start (spot IV). When compared with the experimental embryos, extracts from the control group, without indoleacetic acid, sometimes showed a not very intense spot of β-indoleacetic acid (spot I), always an intense spot II of R_F 0·71, sometimes a weak spot IV at the start. Spot III of R_F 0·05 was never present.

We attempted to compare spot II with a synthetic sample of β-indoleacetamide. The R_F values of spots of indoleacetamide were, however, clearly distinct in systems S_1 and S_2 from the R_F values of spot II (table I). Similarly, the colouring of spot II with formaldehyde, Salkowski's reagent and partially Ehrlich's reagent was different when compared with the spot of indoleacetamide (table II). When the sap expressed from the embryos is extracted with ethyl acetate the compound responsible for spot II readily passes into the solvent.

The orange-pink fluorescent spot III had the same R_F values in systems S_1, S_2, S_4, S_5, and S_6 as authentic β-indoleacetylaspartic acid (table I). Final

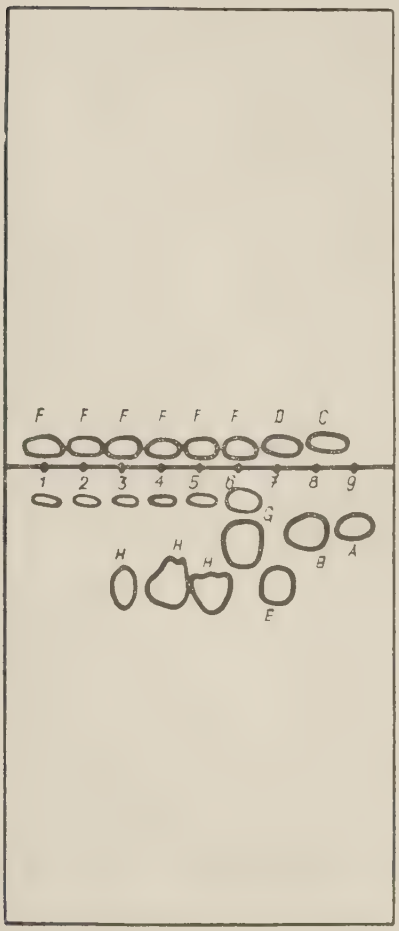

Fig. 1.

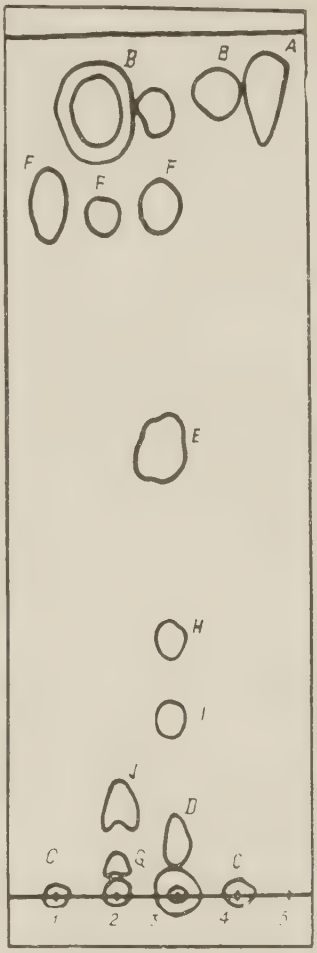

Fig. 2.

Fig. 1. Chromatogram of indole derivatives from extracts of wheat seedlings kept in $3 \cdot 10^{-2}$M indoleacetic acid (2), $3 \cdot 10^{-2}$M tryptophane (3) and from control seedlings (1). (4·5) standards. Detection was carried out with formaldehyde reagent. Spots: **A** β-indolebutyric acid (yellow- brown fluorescence) **B** β-indoleacetic acid (yellow fluorescence spot I); **C** tryptophane (yellow fluorescence — spot IV); **D** malonyltryptophane (orange-pink fluorescence — spot V); **E** unknown substance similar to ascorbigen (orange fluorescence — spot VI); **F** unkown substance (brown- yellow fluorescence — spot II); **G** indoleacetylaspartic acid (orange-pink fluorescence — spot III); **H, I** unknown substances (orange fluorescence); **J** unknown substances (green fluorescence).

Fig. 2. Electrophoreogram of indole derivatives from extracts of wheat seedlings kept in 3 . 10^{-2}M tryptophane (3—5) and from control seedlings (1·2) . (6—9) standards. Electrophoretic division of substances was carried out in phosphate buffer pH 6·68 (conc. M/15), tension 300 V, time of division 4 hours, pontetial declivity 10 v/1 cm.

Detection was carried out by formaldehyde reagent. Spots: **A** β-indolebutyric acid; **B** indolepropionic acid; **C** indoleacetamide; **D** ascorbigen; **E** indoleacetylaspartic acid; **F** tryptophane; **G** β-indoleacetic acid; **H** malonyltryptophane.

Table 1. R_F Values of Indoleacetylaspartic Acid (IAA), Indolelacetamide (IAM) and Spot II in Some Solvents

System	R_F IAA	R_F IAM	R_F Spot II
S_1	0·05	0·64	0·71
S_2	0·79	0·80	0·82
S_4	0·09	0·92	0·91
S_5	0·07	0·79	0·89
S_6	0·27	0·92	0·95

proof of the identity of this spot as indoleacetylaspartic acid was obtained following elution of the spots and their alkaline hydrolysis. To establish the presence of β-indoleacetic acid, spot III was eluted from the chromatogram in system S_1 (R_F 0·05), in which the R_F of free β-indoleacetic acid is 0·86: chromatography of hydrolysates in system S_3 demonstrated the presence of β-indoleacetic acid.

The presence of aspartic acid in hydrolysates of spot III was confirmed following elution of this spot from a chromatogram run in system S_2 (R_F 0·79), hydrolysis, and repeated chromatography.

B. Experiments with tryptophane

On chromatograms of alcohol extracts of embryos kept in $3 \cdot 10^{-2}$M solutions of tryptophane the following spots were always obtained in system S_1 after detection with the formaldehyde reagent:

a) a spot of R_F 0·71 showing intense brownish-yellow fluorescence (spot II)
b) a spot of R_F 0·08 showing orange-pink fluorescence (spot V)
c) a yellow fluorescent spot of tryptophane at the start (spot IV).

In an experiment with a series of nutrient solutions containing graded concentrations of tryptophane ($3 \cdot 10^{-2}$M, 10^{-2}M, 10^{-4}M, 10^{-6}M) the general pattern of the spots did not change, only spots IV and V decreased in intensity.

The chromatographic patterns shown by embryos of the control group without tryptophane have already been described in the foregoing section.

Table 2. Colouring of Spots of Indoleacetylaspartic Acid (IAA), Indoleacetamide (IAM) and Spot II with Some Reagents

Reagent	IAA	IAM	Spot II
Formaldehyde daylight	Y	OB	B
UV radiation	OP	O	BY
Salkowski's	Y	R	RB
Ehrlich's	V	YB	Y, sometimes YB
Cinnamic aldehyde	O	Y	Y

Key: R—red, V—violet, B—brown, O—orange, P—pink, Y—yellow

In the first experiment with tryptophane a further clear spot of R_F 0·52, showing orange fluorescence (spot VI) was found in the chromatogram of extracts from the embryos. The R_F of this spot in systems S_1 and S_2 agreed with the R_F value recorded for ascorbigen. Similarly, the orange fluorescence obtained after detection with the formaldehyde reagent and the blue colour with Ehrlich's reagent are analogous to the reactions of ascorbigen (Procházka 1957). In the course of further experiments it was not, however, possible to confirm the presence of this substance; nor did ascorbigen occur in any of the control experiments.

The substance responsible for spot V shows chromatographic properties similar to those of indoleacetylaspartic acid. Its R_F was practically the same as that of indoleacetylaspartic acid in systems S_1, S_2, S_5, and S_6 (table I). The electrophoretic mobility of the substance producing spot V was also the same as that of indoleacetylaspartic acid. On extraction of the expressed sap of the embryos with ethyl acetate only a small part of this substance passes into the solvent. According to a personal communication from Dr. Andreae a complex compound, malonyltryptophane is formed in plant tissues from exogenous tryptophane. The chromatographic properties of malonyltryptophane as described by him, are similar to those of indoleacetylaspartic acid. Hydrolysis of the complex with barium hydroxide was carried out in eluates of the zone cut from the electrophoreogram, where spot V is sufficiently separated from tryptophane. No indoleacetic acid was present in the hydrolysate. Detection with ninhydrin revealed intense spots in the aspartic acid - glutamic acid region in addition to the tryptophane spot. Electrophoresis of glutamic and aspartic acids is nearly as great as the mobility of the compound responsible for spot V, which may also explain their presence in the hydrolysate. We have assumed that the second component of the complex — malonic acid—is decomposed in the course of hydrolysis. This is also indicated by the result of the control experiment, in which a sample of malonic acid was heated with barium hydroxide under similar conditions. It was not possible to demonstrate malonic acid chromatographically after this treatment.

Discussion

Research into the metabolism of indole substances in plants has recently been advanced by discoveries of new, relatively low molecular indole complexes. Their physiological significance is not yet clear, it may be expected, however, that the discovery of these substances will largely contribute to our knowledge of the metabolism of indole stimulators in plants.

The presence of indoleacetylaspartic acid following the administration of exogenous indoleacetic acid has been described by Andreae et al. (Good, Andreae and van Ysselstein 1956) in a number of plants. According to our results it is possible to add wheat to these. Indoleacetylaspartic acid appears in plants only following the exogenous addition of indoleacetic acid and there is no report in the literature of its occurrence without external intervention. Our results are in agreement with this statement.

In addition to indoleacetylaspartic acid and malonyltryptophane discovered by the Canadian authors, further indole complexes have been described in the recent literature. They are a labile complex growth substance described by RAADTS and SÖDING (1957) in the coleoptiles of oats, and a correlation inhibitor from the roots of peas studied by LIBBERT (1955). A natural low molecular complex, the structure of which is so far best known, is ascorbigen. According to PROCHÁZKA, ŠANDA and ŠORM (1957) ascorbic acid is bound in it to 1-β-indolyl-propen-(2)-diol-(2, 3). From the results of one of us (KUTÁČEK, VALENTA, ICHA 1957 and KUTÁČEK et al. 1958) it follows that ascorbigen is very probably involved in the metabolism of indole growth substances. The presence of ascorbigen in plants other than members of the genera *Brassica* and *Raphanus* has not, however, been demonstrated as yet. The isolated occurrence of a spot of a substance similar in chromatographic properties to ascorbigen in one of our experiments with exogenous tryptophane was, therefore, all the more interesting. For as yet unexplained reasons it has not been possible to repeat this finding. It is possible that it depends on summer conditions. The positive result was obtained in August 1956.

Conjugated derivatives of indole compounds have also been demonstrated in pathological human urine (also indoleacetylglutamine) (JEPSON 1956).

Our work is methodically related to research on ascorbigen. For identification of the metabolites of exogenous indoleacetic acid and tryptophane we used some less usual chromatographic systems, both neutral and acid. Neutral and acid solvents were found to be indispensable for research on ascorbigen, which decomposes in the ammoniacal systems usually used for the separation of indoles (KUTÁČEK, ICHA, VALENTA, TUPÝ and HOLKOVÁ 1958).

Extracts were prepared simply, to prevent by rapidity in procedure the formation of artefacts. The preparation of these simple extracts is, however, inadequate for the demonstration of some substances which are present in very small quantities.

We turned our attention to spots of indole derivatives which appeared on chromatograms in system S_1 close to the start. It was shown that in the case of exogenous indoleacetic acid the spot is caused by indoleacetylaspartic acid, both components of which — indoleacetic acid and aspartic acid — were obtained after hydrolysis. According to a personal communication from ANDREAE and GOOD (cf. DANNENBURG and LIVERMAN 1957), a complex compound — malonyltryptophane — occurs with exogenous tryptophane. Analysis of hydrolysates of this spot on chromatograms in system S_1 showed a bound form of tryptophane. The second component of the complex — malonic acid — could not be demonstrated in view of its lability to hydrolysis.

The results obtained by the method described here, which is different from that used by the Canadian authors, are in full agreement with the results of ANDREAE et al. (ANDREAE and GOOD 1955, GOOD, ANDREAE and VAN YSSELSTEIN 1956) and supplement their observations on other biological material.

We wish to express our thanks to Dr. W. A. Andreae, Science Service Laboratory, University, Sub Post-Office, London—Ontario, for providing us with samples of indoleacetylaspartic acid and for information which he kindly gave us during the course of this work.

References

Abrams, G. J. von: Auxin relations of a dwarf pea. — Plant Physiol. **28** : 443—456, 1953.

Andreae, W. A., Good, N. E.: The formation of indoleacetylaspartic acid in pea seedlings. — Plant Physiol. **30** : 380—382, 1955.

Andreae, W. A., van Ysselstein, M. W. H.: Studies on 3-indoleacetic acid metabolism III. The uptake of 3-indoleacetic acid by pea epicotyls and its conversion to 3-indoleacetylaspartic acid. — Plant Physiol. **31** : 235—240. 1956.

Dannenburg, W. N., Liverman, J. L.: Conversion of tryptophan-2-C^{14} to indoleacetic acid by water-melon tissue slices. — Plant Physiol. **32** : 263—268, 1957.

Good, N. E., Andreae, W. A., van Ysselstein, M. W. H.: Studies on 3-indoleacetic acid metabolism II. Some products of the metabolism of exogenous indoleacetic acid in plant tissues. — Plant Physiol. **31** : 231—235, 1956.

Gordon, S. A., Nieva, S. F.: The biosynthesis of auxin in the vegetative pineapple. I. Nature of the active auxin. — Arch. Biochem. **20** : 356—366, 1949. II. The precursors of indoleacetic acid. — Arch. Biochem. **20** : 367—385, 1949.

Gordon, S. A.: Occurrence, formation and inactivation of auxins. — Ann. Rev. Plant Physiol. **5** : 341—378, 1954.

Hemberg, T.: Studies on the balance between free and bound auxin in germinating maize. — Physiol. Plant. **8** : 418—432, 1955.

Jepson, J. B.: Indolylacetyl-glutamine and other indole metabolites in Hartnup disease. — Biochem. J. **64** : 14 P, 1956.

Kutáček, M., Valenta, M., Icha, F.: Untersuchungen über den Ascorbigengehalt von Kohlrabi (*Brassica oleracea* v. *gongy'odes*) während der Vegetation und den Zusammenhang zwischen Ascorbigen und Wachstum bei den Pflanzen der Familie Brassicaceae. — Experientia **13** : 284—286, 1957; Chem. listy **52** : 537—541, 1958.

Kutáček, M., Icha, F., Valenta, M., Tupý, J., Holková, L.: Studie indolových derivátů v růžičkové kapustě (*Brassica oleracea var. gemmifera*). (A study of indole derivatives in the brussels sprout.) — Chem. listy **52** : 531—536, 1958.

Larsen, P.: Formation, occurrence and inactivation of growth substances. — Ann. Rev. Plant Physiol. **2** : 169—198, 1951.

Libbert, E.: Nachweis und chemische Trennung des Korrelationshemmstoffes und seiner Hemmstoffvorstufe. — Planta **45** : 405 –425, 1955.

Libbert, E.: Die Hydrolyse des „Korrelationshemmstoffes" zu Auxin. — Planta **46** : 256—271, 1955.

Maclachlan, G. A., Waygood, E. R.: Kinetics of the enzymatically-catalyzed oxidation of indoleacetic acid. — Canad. J. Biochem. Physiol. **34** : 1233—1250, 1956.

Petrů, E., Řetovský, R.: Rostlinné explantáty. (Plant tissue cultures.) Praha 1956.

Pilet, P. E.: Action de l'acide β-indolyl-acétique du DL-tryptophane et de l'hydrazide maléoque sur la croissance et le teneur en auxines des racines. — Bull. Soc. Bot. Suisse **66** : 26—38, 1956.

Procházka, Ž., Šanda, V., Šorm, F.: O struktuře askorbigenu. (On the structure of ascorbigen.) — Chem. listy **51** : 1197—1198, 1957; Collection Czechosl. chem. Comm. **22** : 654—655, 1957.

Raadts, E., Söding, H.: Chromatographische Untersuchungen über die Wuchstoffe der Haferkoleoptile. — Planta **49** : 47—60, 1957.

Ray, P. M.: The destruction of indoleacetic acid II. Spectrophotometric study of the enzymatic reaction. — Arch. Biochem. Biophys. **64** : 193—216, 1956.

Siegel, S. M., Galston, A. W.: Experimental coupling of indoleacetic acid to pea root protein in vivo and in vitro. — Proc. Nat. Acad. Sci. US. **39** : 1111—1118, 1953.

Skoog, F.: A deseeded Avena test method for small amounts of auxin and auxin precursors. — J. Gen. Physiol. **20** : 311—334, 1937.

Tang, L. W., Bonner, J.: The enzymatic inactivation of indoleacetic acid. I. Some characteristics of the enzyme contained in pea seedlings. — Arch. Biochem. **13** : 11—25, 1947.

Valenta, M., Kutáček, M., Icha, F.: Stanovení derivátů indolu v rostlinném materiálu. I. Kolorimetrické stanovení askorbigenu a jeho obsahu v některých druzích zeleniny. (Determination of indole derivatives in plant material. I. Colorimetric determination of ascorbigen and its content in some species of vegetables.) — Sborník ČSAZV. Rostl. výr. **29** : 193—202, 1956.

Wildman, S. G., Ferri, M. G., Bonner, J.: The enzymatic conversion of tryptophan to auxin by spinach leaves. — Arch. Biochem. **13** : 131—144, 1947.

Wildman, S. G., Muir, R. M.: Observations on the mechanism of auxin formation in plant tissues. — Plant Physiol. **24** : 84—92, 1949.
Wildman, S. G., Gordon, S. A.: The release of auxin from isolated leaf proteins of spinach by enzymes. — Proc. Nat. Acad. Sci. US **28** : 218—228, 1942.
Wildman, S. G., Bonner, J.: The protein of green leaves. I. Isolation, enzymatic properties and auxin content of spinach cytoplasmic proteins. — Arch. Biochem. **14** : 381—413, 1947.

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К вопросу превращений вводимых извне триптофана и β-индолилуксусной кислоты в изолированных зародышах пшеницы

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Резюме

Для выяснения обмена триптофана и β-индолилуксусной кислоты прибавляли к изолированным зародышам пшеницы, культивируемым в растворе Кнопа, или триптофан, или β-индолилуксусную кислоту в концентрации $3 \cdot 10^{-2}$ М. Через 24 часа из зародышей быстро приготовляли спиртовые экстракты, которые анализировали хроматографически или электрофоретически. Кроме хроматографических систем и проявителей применяемых обычно при анализе соединений индола, использовались также некоторые менее известные системы, оправдавшие себя при анализе не давно открытого деривата индола у растений семейства *Brassicaceae* — аскорбигена. Это системы: бутилацетат, насыщенный водой: тетрахлорметан — уксусная кислота (100 : 2); формальдегидный проявитель (40% формалдегид—концентрированная HCl—вода 1 : 1 : 2). Электрофорез на бумаге производился в М/15 фосфатном буфере при pH 6,6 и 300 V в течение 4—5 час. Главное внимание посвящалось доказательству гидрофильных комплексов вводимых веществ. В зародышах, выживающих в растворах β-индолилуксусной кислоты, кроме других дериватов индолила, было установлено присутствие β-индолилуксусноаспарагиновой кислоты. Она была идентифицирована по R_F в 5 различных системах с помощью цветных реакций и хроматографического доказательства наличия ее компонентов после щелочного гидролиза (6 N $Ba(OH)_2$ при 100° С, 30 мин.). В зародышах, выживающих в триптофане, было найдено вещество с такими же хроматографическими свойствами, как индолилуксусноаспарагиновая кислота, которое однако после гидролиза давало триптофан. По-видимому, это малонилтриптофан. Кроме того в одном опыте наблюдалось возникновение вещества, хроматографически сходного с аскорбигеном. — Настоящая работа подтверждает с помощью других средств и на другом биологическом материале результаты, которые одновременно получили Andreae и Good.

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Untersuchungen über die durch Ultraschall hervorgerufenen Sofortveränderungen in der Zelle

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Souhrn

V práci se jedná o průkaz specifických změn vyvolaných ultrazvukem v epidermis *Allium cepa*. V dřívějších pracích byly prováděny rozbory účinku ultrazvuku až po proběhnutí sekundárních biologických pochodů, kde bylo zjištěno, že se po ozvučení nevyskytují jiné stavy buněk než buňky živé nebo mrtvé. Analysa okamžitého účinku ultrazvuku vede však k závěru, že můžeme nalézt několik stavů poškození.

Pro zachycení různých stavů změn buněčné permeability byla zvolena metodika fluorescenční mikroskopie s použitím barvení akridinovou oranží. Je prokázána možnost použití tohoto barviva jako barviva vitálního současně s možností posouzení stavu buňky podle barevných rozdílů. Blána buněčná fluoreskuje žlutě, protoplasma živých buněk žlutozeleně, zatím co protoplasma odumřelých buněk fluoreskuje jasnou cihlově červenou barvou. Tento přesun optického těžiště se vysvětluje na základě změny elektroadsorptivních sil v micelární struktuře bílkoviny. Cytologicky je možno zjistit řadu změn, které jinou metodou by nebyly prokazatelné. Okamžitý účinek ultrazvuku, projevující se v koloidně osmotických změnách protoplasmy, vzniká zejména změnami permeability. Jedná se o specifický druh koagulace se snížením permeability.

V metodice ozvučení bylo použito ultrazvukového terapeutického generátoru VEM US 2-1 fy Röntgen u. Transformatorenwerk Dresden s frekvencí 0,8 Mc/sec a měnitelnými intensitami 0,2—4 W/cm². Dopadající ultrazvuková energie byla zvlášť proměřena, jak je uvedeno v jiné práci.

Jednotlivé stavy poškození byly pozorovány jednak ze změn optického těžiště a jednak ze změn tvarových. Buňky, které vliv přežily, vykazují normální fluorescenci. První změny v protoplasmě celé buňky se projeví zvýšením fluorescence jádra a současně se vyznačí i drobné změny v cytoplasmě, která jeví flokulaci. Optické těžiště fluorescence však není ještě změněno. Těžší změny se ukazují jako hrubší změny na jádře, kde není ještě změněno optické těžiště, ale současně jsou hrubší změny v cytoplasmě, která porušením své micelární struktury mění i elektroadsorptivní síly a dochází ke změně barvy fluorescence. U buněk, které podlehly hrubým změnám, dochází k hrubé koagulaci protoplasmy se změnami na jádře, což se jeví změnami tvarovými i změnami optického těžiště.

Průkaz těchto jednotlivých stavů poškození neznamená, že v sekundárních pochodech biologických nemohou proběhnout pochody, které vedou jen k buňkám mrtvým a živým. Ukazuje se však, že kvantitativní výtěžek ultrazvukové energie působící na buňku může být různý.

Zusammenfassung

Die Autoren bringen eine kurze Mitteilung über Beobachtungen der sofortigen Veränderungen in den Zellen der Epidermis von Allium cepa durch Ultraschallwirkung. Als Beobachtungsmethodik dieser Veränderungen wurde die Fluoreszenzmikroskopie mit Akridinorange-Färbung verwendet. Es werden vier Stadien der hervorgerufenen Veränderungen unterschieden. Auf Grund dieser Beobachtungen wird angenommen, dass die quantitative Ausbeute der Energie des Ultraschalles für biologische Vorgänge verschieden sein kann, auch wenn in den sekundären Vorgängen die Ultraschallwirkung entweder zum Absterben oder Überleben der Zelle führt. Aus zytologischen Beobachtungen wird auf kolloidal-osmotische Veränderungen geschlossen.

Einleitung

Es gibt eine ganze Reihe zytologischer Arbeiten, welche bemüht sind, die mit dem Wirkungsmechanismus des Ultraschalls verbundenen Fragen zu lösen und die wichtigsten Objekte von verschiedenen Gesichtspunkten aus zu bearbeiten. In früheren Versuchen (RAUŠER 1957a) führten wir ebenfalls einige zytologische Analysen durch, mit dem Ziel, die quantitative Ultraschallwirkung allgemein zu analysieren. Bei weiterer Präzisierung der Methodik gelangten wir zu einigen Ergebnissen, welche zu speziellen Schlussfolgerungen führen könnten — besonders hinsichtlich der eigentlichen Erkenntnise der Ultraschallwirkung auf die lebende Zelle. Diese Resultate sind der Inhalt unserer kurzen Mitteilung.

Im wesentlichen handelte es sich uns um den Nachweis der spezifischen Veränderungen, welche mittels Ultraschall an den Zellen der Epidermis von *Allium cepa* hervorgerufen werden. Bereits in früheren Studien kamen wir wie andere Autoren (HERČÍK, HRDLIČKA u. ŠPRINDRICH 1946) zu dem Schluss, dass es sich um kolloidal-osmotische Veränderungen handelt, welche zur Retraktion und Desintegration des Protoplasmas und irreversibel zum Zelluntergang führen. Wenn wir die Ultraschallwirkung auf die Zelle der Epidermis von *Allium cepa* erst nach Ablauf der sekundären biologischen Vorgänge einer Analyse unterziehen, so können wir feststellen, dass nach der Exposition der Zellen keine anderen Stadien vorkommen, als lebende oder tote Zellen. Die Analyse der augenblicklichen Ultraschallwirkung führt allerdings zu dem Schlusse, dass einige Übergangsstadien der Beschädigung vorkommen. Die bisherigen Beobachtungen stimmen im allgemeinen mit den früheren Arbeiten von LEHMANN und FINSTERWALDER (1948), HLUCHOVSKÝ und PÚŽA (1951) sowie von HARWEY und LOOMIS (1931) u. a. überein. Unsere Versuche ergänzen frühere Arbeiten und Beobachtungen dahingehend, dass die Ultraschallwirkung mit grosser Wahrscheinlichkeit ein Analogon der Desintegration durch ionisierende Strahlung ist. Die kolloidalen Veränderungen in der Zelle nach der Ultraschallwirkung sind allerdings verschieden, u. zw. sowohl zeitlich, als auch qualitativ, wodurch sich die Eigenart dieser physikalischen Energie offenbart.

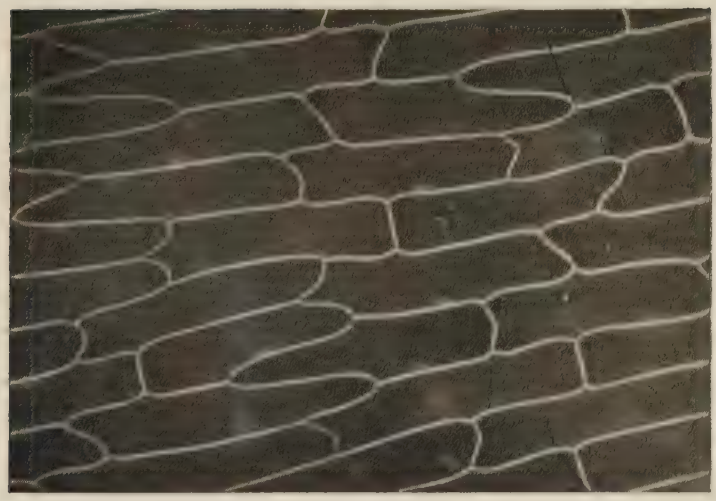

Abb. 5. Mikrofotografie der unbeschädigten Epidermis.

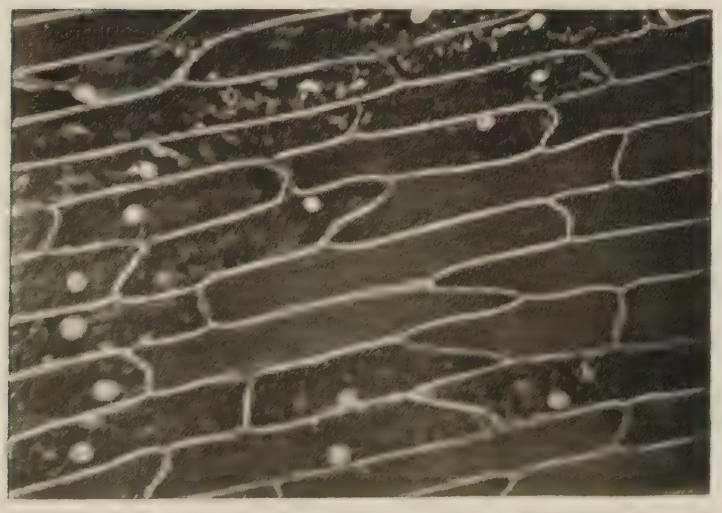

Abb. 6. Mikrofotografie der beschallten Epidermis.

Bei den früheren Untersuchungen über die Ultraschallwirkung auf die Epidermis von *Allium cepa* wurden verschiedene Testarten verwendet. Die Methode der Supravitalfärbung mit Erythrosin konnte grösstenteils nur die sekundären Veränderungen erfassen. Die Methode der Fluoreszenmikroskopie liess bei Verwendung der Supravitalfärbung mit Kaliumfluoreszein nicht genügend deutlich die verschiedenen Stadien der Veränderungen der Zellpermeabilität erkennen. Wir hielten deshalb die Methode der Fluoreszenmikroskopie unter Anwendung der Färbung mittels Akridinorange für die vorteilhafteste.

Akridinorange als basisches Fluorochrom wurde von STRUGGER (1941) eingeführt. In einer Reihe früher durchgeführter Experimente wurde die Möglichkeit seiner Verwendung als Vitalfarbstoff zur Bestimmung des Zustandes der lebenden Zelle nach ihren Farbunterschieden, erwiesen. Die Zellmembran fluoresziert gelb, das lebende Protoplasme gelb-grün, während das Protoplasma abgestorbener Zellen in einer klaren ziegelroten Farbe leuchtet. Diese Verschiebung des optischen Schwerpunktes der Fluoreszens ist dadurch zu erklären, dass das lebende Protoplasma den Farbstoff nur mit schwacher elektroadsorptiver Kraft annehmen kann. Ist jedoch die Struktur der Eiweisskörper verändert, besonders im Sinne der kolloidalen Gliederung des Micellen, dann entsteht die Möglichkeit der Verstärkung dieser Verbindung Die Zunahme der Farbmenge geht so weit, dass sich die ursprüngliche Farbe der kleinen, fluoreszierenden Menge von Akridinorange — gelbgrün— in eine ziegel- bis kupferrote Farbe verwandelt. Die Verschiebung des optischen Schwerpunktes der Fluoreszenz ist also mit feinen Veränderungen des Protoplasmas in der kolloidalen Formation verbunden. Es handelt sich aber nicht nur um totale Veränderungen in der Verschiebung der Wellenlänge. Zytologisch kann eine Reihe von Veränderungen in der Annahme des Akridinorange durch verschiedene Strukturen festgestellt werden, welche mit einer anderen Methodik nicht so leicht nachweisbar sind.

Aus früheren Arbeiten und aus unseren eigenen Versuchen kann geschlossen werden, dass die sofortige Ultraschallwirkung, welche sich in kolloidalosmotischen Veränderungen des Protoplasmas äussert, besonders in Veränderungen der Permeabilität in Erscheinung tritt. Dafür sprechen auch Modellversuche, welche durch Ultraschall bewirkte Veränderungen in den Kolloiden, im Sinne der Thixotropie (RAUŠER 1958) nachweisen. Die Beschädigung des Kolloidnetzes in diesem Sinne kann dann zur Koagulation der nativen Eiweissstoffe führen. Die durch Ultraschall hervorgerufene Koagulation des Protoplasmas ist aber nicht dieselbe wie jene Veränderungen, welche z. B. durch einfache Wärmeenergie verursacht sind, wobei die Permeabilität derart erhöht ist, dass sich der ganze Zellinhalt unspezifisch färbt, z. B. durch supravitale Farbstoffe. Es liegt wahrscheinlich hier eine andere Art von Koagulation mit Herabsetzung der Permeabilität vor. Das entspricht auch der Ansicht (HERČÍK, HRDLIČKA u. ŠPRINDRICH 1946), dass es sich um die Bildung besonderer Koagulationsbarrieren handelt, die gleichzeitig mit den nachfolgenden groben Veränderungen in der Struktur des inneren Plasmas erfolgt. Veränderungen am Zellkern – Übergang des hyalinen Stadiums in die pyknotische Anschwellung, später das Aufspringen der Struktur und die Flockung — sind auch von anderen Gesichtspunkten aus

interessant, müssen aber mit Rücksicht auf ihre Unterschiedlichkeit gesondert studiert werden. Dies bestätigen auch die Beobachtungen verschiedener Autoren (Hluchovský u. Půža 1951 u. a.).

Methodik

Als Versuchsobjekt wurde die obere Epidermis des dritten Blattes (gerechnet vom ersten fleischigen Aussenblatt) von *Allium cepa*, Zittauer Abart, mit einem Zwiebeldurchmesser von cca 50—70 mm verwendet. Nach Beseitigung des an der konvexen Seite anhaftenden Mesenchyms wurde die Epidermis in Stücken von cca 10 × 10 mm beschallt. Die Behandlung der Epidermis vor der Beschallung (Beseitigung des Mesenchyms) zeigte sich bei Kontrollversuchen als vital einwandfrei, was bereits durch frühere Arbeiten (Herčík 1939 u. a.) bewiesen wurde.

Die Beschallung wurde genau wie in früheren Versuchen (Raušer 1957a, b) vorgenommen, und zwar so, dass die in einer Klammer aus Pertinax gespannte Epidermis in einem Wasserbad untergebracht wurde, wobei sich die Scheibe mit der Epidermis vor dem Schwinger des Ultraschallgenerators, in konstanter Entfernung und fortpflanzenden Wellen befand.

Benützt wurde der Ultraschallgenerator für therapeutische Zwecke VEM US-2-1 der Firma Transformatoren u. Röntgenwerk, Dresden, welcher mit einer Frequenz von 0·8 Mc/sec und veränderlichen Intensitäten von 0·2—4 W/cm² arbeitet. Die Messungen der Energie des Ultraschalles wurden in einer früheren Arbeit (Raušer 1957b) beschrieben. Gearbeitet wurde im entfernteren Wellenfelde, d. h. im Felde des letzten Interferenzmaximums, um Fehler der Unhomogenität des Feldes aus dem Interferenzminimum zu eliminieren. Die übrigen Einzelheiten der Methodik der Schalleinwirkung sind in den angeführten früheren Arbeiten beschrieben.

Zur zytologischen Analyse wurde die Färbung mit Akridinorange (Merck) 1 : 2000 in Acetatpuffer von pH 4,8 verwendet. Die Dauer der Färbung betrug 20 sec, das Wässern 30 sec. Die Beobachtungen wurden mit dem Zeiss'schen Fluoreszenzapparat mit dem UG-1 Filter Schott und über $CuSO_4$ ausgeführt. Die Färbung und Beobachtung erfolgten sofort nach der Einwirkung. Die Mikrofotografien wurden mit aufgesetzter Fotokamera Pentacon und Agfa-Isopan-Kinofilm mit hellem Orangefilter aufgenommen.

Ergebnisse

Zur Analyse der Einwirkung wurden verschiedene Intensitäten von 1 W/cm², 2 W/cm² u. 3 W/cm² bei verschiedener Expositionsdauer verwendet. Alle Versuche zeigten, dass sich die der Schallwirkung ausgesetzten Präparate lediglich durch die Anzahl der beobachteten Arten der Zellschädigung unterschieden; Unterschiede in der Qualität wurden nicht beobachtet.

Die einzelnen Grade der Zellschädigung wurden einerseits in den Veränderungen des optischen Schwerpunktes der Fluoreszenz, andererseits in den Formveränderungen festgestellt. Nach dem Einwirken der Ultraschallwellen unter unseren Versuchsbedingungen konnten wir folgende Stadien der Beschädigung unterscheiden.

I. Lebende Zellen. Bei diesen Zellen ist die Fluoreszenz bis auf die Zellmembran grösstenteils matt. Die Zellmembran fluoresziert deutlich orange, wodurch die Grenzen der einzelnen Zellen genau bestimmt sind. Das Protoplasma färbt sich gelbgrün und läst einen schwach fluoreszierenden Zellkern von normaler Grösse erkennen, in welchem keine Struktur wahrnehmbar ist (Abb. 1).

II. Beschädigte Zellen. Veränderungen, welche in diesem Stadium der Beschädigung der Zellen erkennbar sind, erweisen sich als Veränderungen im

Protoplasma und im Zellkern. Im Protoplasma, dessen Farbe sich von jener der lebenden Zellen nicht unterscheidet, findet sich eine kleinere Menge Koagulat, welches sich grün färbt. Der Zellkern fluoresziert lichtgrün, so dass die Grenze zwischen ihm und dem Protoplasma deutlich bestimmbar ist. Darin sind Körner sichtbar, welche sich deutlich gelb färben. An der Peripherie des Zellkernes beginnt die Koagulation des Karyoplamas, welche in einer satteren grünen Farbe fluoresziert, als der übrige Teil des Kerns (Abb. 2).

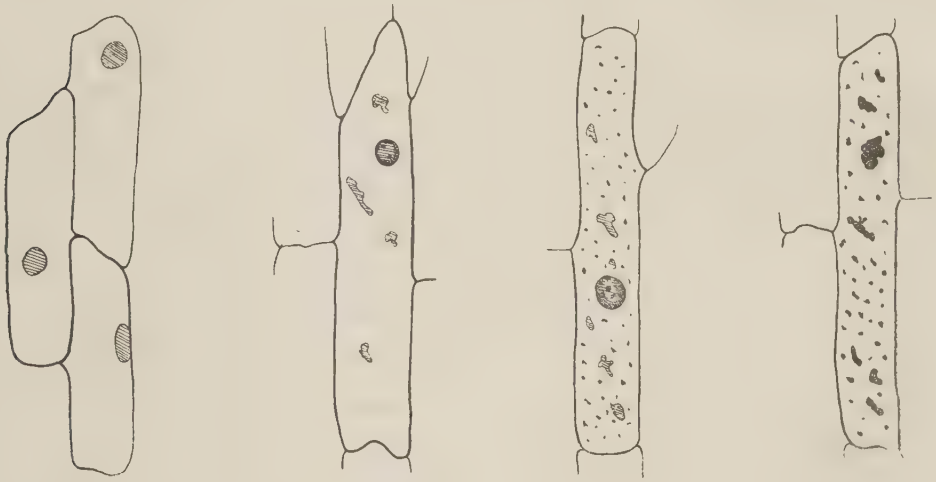

Abb. 1. Abb. 2. Abb. 3. Abb. 4.

Abb. 1. Lebende Zellen, normale Fluoreszenz.
Abb. 2. Beschädigte Zellen, Veränderungen am Zellkern und im Zytoplasma.
Abb. 3. Schwerbeschädigte Zellen, Veränderungen am Zellkern und im Zytoplasma mit gleichzeitiger Veränderung der Fluoreszenz.
Abb. 4. Tote Zellen, schwere Veränderungen am Zellkern und im Zytoplasma.

III. Schwerbeschädigte Zellen. Im Protoplasma der schwerbeschädigten Zellen erscheinen neben groben, grünen Koagulaten auch zarte, rot gefärbte Koagula, welche das Hauptmerkmal dieses Zustandes sind. Das Protoplasma macht den Eindruck, als ob es mit diesen zarten Koagulaten punktiert wäre. Der Zellkern ist lichtgrün und zeigt scharf fluoreszierende Körnchen. Das Karyoplasma, welches im vorigen Stadium der Beschädigung nur am Rande des Zellkernes koagulierte, beginnt nun vom Rande aus mehr zur Mitte zu koagulieren. Der Kernrand, welcher bei beschädigten Zellen grün verfärbt war, nimmt jetzt eine Orangefärbung an. Abbildung 3. illustriert diesen Zustand der Beschädigung schematisch.

IV. Tote Zellen. Zum Unterschied von lebenden Zellen ist bei den toten Zellen keine grüne Fluoreszenz zu bemerken. Die Zellmembrane färbt sich hellorange. Im Protoplasma finden sich, grobe und feine rote Koagulate.

Der Zellkern ist rot mit grob koaguliertem Karyoplasma, so dass er oft einer Maulbeere gleicht; Nucleolen sind nicht zu unterscheiden (Abb. 4).

Als Beispiel der Beobachtungen mögen Mikrofotografien der unbeschädigten Epidermis dienen (Abb. 5). Die Farbunterschiede kann die schwarzweisse Fotografie allerdings nicht erfassen. Zur Illustration einer der Schallwirkung ausgesetzten Epidermis bringen wir die Mikrofotografie (Abb. 6) eines Präparates, welches der Schallwirkung mit einer Intensität 3 W/cm^2 bei einer Expositionsdauer von 3 Min. ausgesetzt war. Die Stadien von unbeschädigten Zellen, beschädigten und schwerbeschädigten sowie toten Zellen sind erkennbar.

Diskussion

Die einzelnen Stadien der Zellbeschädigung sind aus der sofortigen Einwirkung des Ultraschalles erklärbar, höchstwahrscheinlich auf Grund des folgenden Vorganges. Bei Zellen, welche groben Veränderungen unterlagen, kam es unmittelbar zu grober Koagulation des Protoplasmas mit gleichzeitiger Veränderung am Zellkern, was folgerichtig Veränderungen des optischen Schwerpunktes des Protoplasmas, sowie Veränderungen des Zellkernes und des Plasmas nach sich zog. Zellen, welche die Einwirkung überlebten, zeigten die normale Fluoreszenz eines Gewebes, welches nicht dem Ultraschall ausgesetzt war. Je nach dem Stande der Beschädigung kann geschlossen werden, dass die ersten Veränderungen im Protoplasma der ganzen Zelle sich durch erhöhte Fluoreszenz des Zellkernes manifestieren (Stadium der Beschädigung); gleichzeitig machen sich kleine Veränderungen im Zytoplasma bemerkbar, welches eine Flockung aufweist. Der optische Schwerpunkt der Fluoreszenz ist allerdings nicht verändert, was beweist, dass die elektroadsorptiven Kräfte noch nicht verändert sind. Schwere Veränderungen (Stadium der schweren Beschädigung) erweisen sich als gröbere Veränderungen des Zellkernes, wobei aber der optische Schwerpunkt der Fluoreszenz noch nicht verändert ist. Gleichzeitig entstehen gröbere Veränderungen im Zytoplasma, welches infolge der Schädigung seiner mizelaren Struktur seine elektroadsorptiven Kräfte ändert, wobei die Fluoreszenz ihre Farbe wechselt.

Der Nachweis dieser einzelnen Stadien der Beschädigung bedeutet nicht, dass in den sekundären biologischen Vorgängen nur Veränderungen vorkommen können, welche bloss zu überlebenden oder toten Zellen führen, wie dies frühere Versuche ergaben. Es zeigt sich aber, dass die quantitative Ausbeute der Ultraschallwirkung auf die Zelle verschieden sein kann. Hier spielt bestimmt eine ganze Reihe von physikalischen und biologischen Faktoren eine Rolle.

Vom Gesichtspunkte des Wirkungsmechanismus aus wird in verschiedenen Stadien der momentanen Beschädigung der Beweis früherer Beobachtungen und Hypothesen erbracht, dass es von den primären physikalischen Einwirkungen (mechanische Energie des Ultraschalls) über physikalisch-chemische Vorgänge (kolloidale und kolloidal-osmotische Veränderungen) zur eigentlichen biologischen Kundgebung — zum Zelltod — dem Tod der Zelle kommt. Eine genauere Analyse des Mechanismus der einzelnen Vorgänge würden den Rahmen dieser Mitteilung übersteigen.

Literatur

Harwey, E. N., Loomis, E. B.: High speed photomicrography of living cells subjected to supersonic vibration. — J. gen. Physiol. **15** : 147—154, 1931.
Herčík, F.: Die fluoreszenzmikroskopische Analyse der α-Strahlenwirkung. — Protoplasma **32** : 527—538, 1939.
Herčík, F., Hrdlička, M., Šprindrich J.: Biologický účinek ultrazvuku. (Die biologische Ultraschallwirkung.) Sborník lékařský **68** : 1—8, 1946.
Hluchovský, B., Půža, J.: Koloidní změny v buňkách vyvolané ultrazvukem. — (Kolloidale Veränderungen in den Zellen, hervorgerufen durch Ultraschall.) — Biologické listy **32** : 148 — 158, 1951.
Lehmann, J., Finsterwalder, G.: Die Koagulationswirkung des Ultraschalles. — Arch. exp. Path. Farm. **206** : 314—321, 1948.
Raušer, V.: Biologický účinek ultrazvuku. (Die biologische Ultraschallwirkung.) — Diss. ČSAV Brno 1957a.
Raušer, V.: Měření energie ultrazvuku a jeho použití v biologii a therapii. (Messungen der Ultraschallenergie und ihre Verwendung in Biologie und Therapie.) — Babákova sbírka **3**, Praha 1957b.
Raušer, V.: Thixotropický účinek ultrazvuku. (Die thixotropische Wirkung des Ultraschalles.) — Spisy přírodov. fakulty MU Brno 1958.
Strugger, S.: Zellphysiologische Studien mit Fluoreszenzindikatoren. — Flora **35** : 101—113, 1941.

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Исследования непосредственных изменений в клетке под влиянием ультразвука

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Резюме

Речь идет о доказательстве наличия специфических изменений, вызываемых ультразвуком в эпидермисе *Allium cepa* L. В прежних работах был произведен анализ действия ультразвука только после осуществления вторичных биологических процессов, причем было установлено, что после воздействия ультразвука не наблюдается никаких других состояний клеток, кроме живых или мертвых клеток. Однако анализ непосредственного действия ультразвука показывает, что можно отметить несколько различных состояний повреждения.

Для определения различных состояний изменения клеточной проницаемости была избрана методика флюоресцентной микроскопии с применением окраски акридиновым оранжевым. Была доказана возможность использовать это вещество в качестве прижизненного красителя для одновременной оценки состояния клетки по различиям в окрашиваемости. Клеточная оболочка флюоресцирует желтым цветом, протоплазма живых клеток — желтозеленым, тогда как протоплазма отмерших клеток — ярким кирпично-красным цветом. Это смещение оптического центра тяжести объясняется на основании изменения электроадсорбтивных сил в мицеллярной структуре белка. Цитологически можно установить ряд изменений, наличие которых другими методами невозможно было бы доказать.

Непосредственное действие ультразвука, проявляющееся в коллоидно-осмотических изменениях протоплазмы, возникает, главным образом, в результате изменений проницаемости. Речь идет о специфической форме коагуляции с понижением проницаемости.

Что касается методики применения ультразвука, использовался терапевтический ультразвуковой генератор VEM US 2—1 фирмы Röentgen u. Transformatorenwerk,

Dresden, с частотой 0,8 Мс/сек. и с интенсивностью, могущей меняться в пределах 0,2—4,0 W/см². Попадающая на объект энергия ультразвука была измерена отдельно, как показано в другой работе.

Отдельные состояния нарушения оценивались по изменениям оптического центра тяжести и по морфологическим изменениям. В клетках, которые выживали после воздействия ультразвука, наблюдалась нормальная флюоресценция. Первые изменения в протоплазме всей клетки проявляются в повышении флюресценции ядра, а одновременно наблюдаются небольшие изменения в цитоплазме, в которой появляется флокуляция, но оптический центр тяжести флюоресценции еще не меняется. Более тяжелые нарушения проявляются как значительные изменения ядра, где оптический центр тяжести однако еще не меняется, а одновременно как более резкие изменения в цитоплазме, где в результате нарушения мицеллярной структуры меняются и электроадсорбтивные силы и наблюдается изменение цвета флюоресценции. У клеток, которые подверглись глубоким изменениям, наблюдается сильная коагуляция протоплазмы с изменениями в ядре, что отражается и на изменениях формы и смещении оптического центра тяжести.

Открытие всех этих стадий нарушения не означает, конечно, что при вторичных биологических процессах не могут наступить состояния, которые в результате дают или мертвые или живые клетки. Оказывается однако, что окончательное количество энергии ультразвука, действующей на клетку, может быть различно.

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Humic Acids with C^{14}

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Souhrn

1. Listy cukrovky *(Beta saccharifera)*, jež asimilovaly aktivní kysličník uhličitý $C^{14}O_2$, byly v zemině podrobeny pochodu humifikace. Po třech až pěti měsících humifikace byla usušená zemina extrahována ether-alkoholem, pak natrium nebo kaliumhydroxydem a kyselinou chlorovodíkovou byly huminové kyseliny odděleny od fulvokyselin. Všecky frakce byly radioaktivní.

2. Rostliny kukuřice *(Zea mays)* vyrostlé ve zředěném Knopově živném roztoku s přidanou aktivní huminovou kyselinou jevily v kořenech i v listech radioaktivitu. Mezi kořeny a listy však byl velký rozdíl. Byla-li aktivita kořenů po dvou až dvanácti dnech asi 100 až 200 imp./min, byla aktivita listů kolem 20 imp./min počítáno na jednu rostlinu nebo asi 300 imp./min/10 mg u kořenů a na hranici měřitelnosti u listů.

3. Když na spodní část rubu prvního listu kukuřice byla nanesena kapka roztoku a suspense aktivní kyseliny huminové, šířila se velmi málo i v tomto listu; aktivita druhého a třetího listu byla i po pěti dnech na hranici měřitelnosti; obdobně v kořenech. Také z kapilár zabodnutých do mesokotylu se kyselina huminová šířila obdobně, po pěti dnech byla na hranici měřitelnosti.

4. Autoradiogramy se plně shodovaly s výsledky získanými počítačem. Kořeny byly prokresleny zřetelně a více méně intensivně, kdežto listy se neobjevily nebo jen jako slabé stíny.

5. Poněvadž aktivita živného roztoku během kultivace stoupla, nepřijaly rostliny ani to množství huminové kyseliny, jež bylo rozpuštěno v objemu vytranspirované vody; z toho snad lze soudit, že rostliny přijímaly velké molekuly, a proto pomalu. Rozhodně nelze mluvit o hromadění v pletivu.

6. Naše pokusy nerozhodují, proniká-li nezměněná kyseliny huminová do buňky. Můžeme podle nich pouze uzavírat, že, přidáme-li radioaktivní huminovou kyselinu do vody nebo do živného roztoku, objeví se radioaktivita v kořenech a později slabě i v listech. Připustíme-li že v rostlinných orgánech naměřená aktivita byla působena aplikovanou kyselinou huminovou přímo, znamená to, že proniká do rostliny pomalu, není hromaděna a z kořenů se po rostlině šíří pomalu.

Je možné, že se radioaktivní uhlík dostane do rostliny v molekule kyseliny huminové, není však vyloučena možnost, že se do kořenů a z kořenů do listů dostane teprve po rozkladu velké molekuly, eventuálně teprve po její mineralisaci. Ale při rychlé mineralisaci by se v rostlině jistě šířila rychleji.

Summary

1. Leaves of sugar beet *(Beta saccharifera)*, which had assimilated active carbon dioxide $C^{14}O_2$, were subjected to the process of humification in soil. After three to five months of humification the dried soil was extracted with

ether-alcohol, then with sodium or potassium hydroxide; the humic acids were separated from fulvic acids with hydrochloric acid. All fractions were radioactive.

2. Maize plants *(Zea mays)* grown in diluted Knop's solution with the addition of active humic acid showed radioactivity in the roots and leaves. There was, however, a marked difference between the roots and the leaves. While the activity of the roots after two to twelve days was about 100 to 200 cpm., that of the leaves was about 20 cpm. as calculated for one plant, or about 300 cpm./10 g. for roots and at the limits of measurement for leaves.

3. When a drop of solution and suspension of active humic acid was placed on the lower surface of the first leaf, it spread very little even in this leaf; the activity of the second and third leaves was at the limits of measurement after five days; the same applies to the roots.

4. Autoradiograms were fully in keeping with the results obtained with the counter. Roots were clearly marked and more or less intensely, while the leaves did not appear or appeared only as faint shadows.

5. These experiments do not resolve the question of whether unchanged humic acid penetrates into the cells. It can only be concluded, on the basis of these experiments, that if radioactive humic acid is added to water or to nutrient solution, radioactivity appears in the roots and later weakly in the leaves. If it is assumed that the activity measured in the plant organs was caused by humic acid applied direct, this means that it penetrates into the plant slowly, it is not accumulated and spreads throughout the plant slowly from the roots.

Preparation of active humic acids

As the source of active material we used sugar beet *(Beta saccharifera)* leaves extracted with water. They assimilated active carbon dioxide $C^{14}O_2$ and, in the water extract and in the non-dissolved residue (Liebster, Chrastil and Babický 1957), contained both radioactive assimilates and anabolic substances formed from them. These were subjected to the normal course of humification. Thus, labelled humic acids could be formed both by the humification of the radioactive compounds contained in the beet leaf, and also, through the exchange of active carbon with the carbon of humic acids already contained in the mixture with earth. We did not deal with this question, which had no essential effect on the result of the experiment. Still less is it possible as yet to judge in what group and in what manner active carbon is bound. In the meantime, however, it may be regarded as decisive that the humic acid fraction prepared in the usual way, i. e. by alkali extraction and precipitation with acid, and the other fractions, were radioactive (Prát, Čatský and Melichar 1957, Prát 1958).

As the substrate for humification and as inoculation material we chose leafmold (fine earth sieved through a two millimetre sieve), which did not effervesce in hydrochloric acid. In the second series we therefore omitted the preliminary extraction with hydrochloric acid.

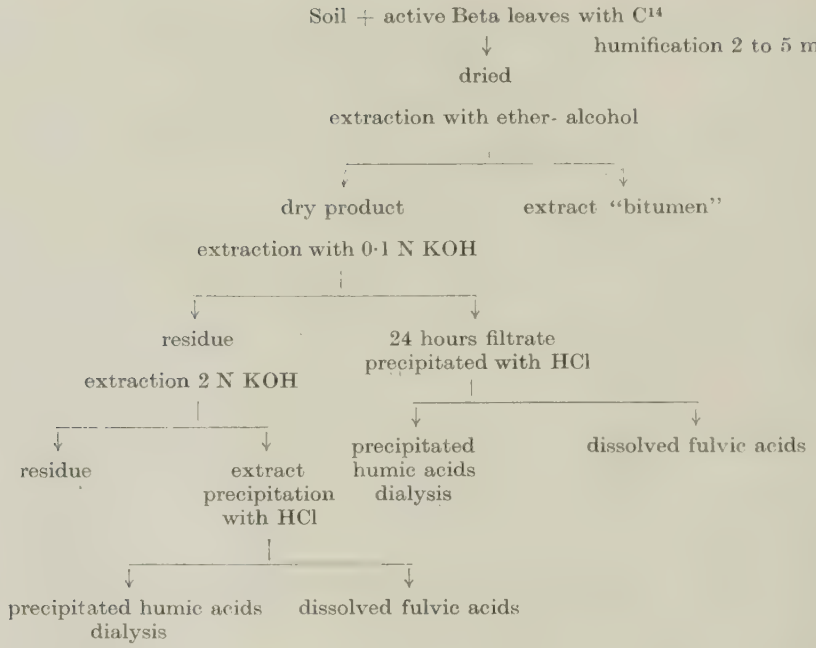
In the first experiment we used about 396 mg. of dry beet leaves with an activity of 394 μC, i. e. about 1 μC/mg., content of C^{14} about 119 mg. We exposed these leaves to humifying conditions in a mixture of 25 g. of dry leafmold, 1·5 g. dry normal beet leaves and 20 ml. of water. The material was mixed and put in a covered dish in a desiccator with 20 ml. 0·1 N NaOH in the dish and test-tube. After sixteen weeks (5. VII to 24. X. 1956) the soil was dried at a temperature of 85° C, extracted with 50 ml. 0·1 N HCl; following filtration it was extracted with 0·1 N NaOH. The filtrate was precipitated with 2 N HCl and on the following day the sediment (brown flakes) was centrifuged. The clear yellow supernatant was active, but dialysis of fulvic acids has hitherto only been partially successful, because they passed through the membranes used. The sediment of humic acids obtained by centrifuging was dried (preparation a). It was again dissolved and precipitated to clean it. The procedure in the second experiment was similar. 10 g. of fresh leafmold sieved through a 2 mm. sieve was mixed with 30 g. of fresh beet leaves, 170 mg. of dry radioactive beet leaves and 6·5 ml. of distilled water were added. Humification proceeded under conditions similar to those of the first experiment. After about 4 months (5. I. to 2. V. 1957) the material was dried at a temperature of 52° C. The dried sample was first extracted in Soxlet's apparatus for 24 hours with 50 ml. ethylalcohol and 50 ml. ethylether. The clear yellow extract later turned greenich; when dried in the air the residue ("bitumen") was dark green.

50 ml. of 0·1 N KOH was poured on to the soil that had been extracted with alcohol-ether, the filtrate was precipitated after 24 hours with HCl and the brown flakes obtained were centrifuged after a further 24 hours. The sediment was purified by dialysis against distilled water and dried at ordinary temperatures (preparation b).

The ratio of the activities of "bitumen", fulvic acids and humic acids to the extracted residue was about 6 : 6 : 10 : 25. This residue of soil was then extracted again with hot 2 N KOH (40 ml.) for about 30 min. It was left to cool, centrifuged on the second day; the supernatant was precipitated with HCl, the second (less soluble) fraction of fulvic acids, humic acids and humin (preparation c) being thus separated; these were considerably more active than those from the first extraction, 0·01 ml. giving 285 cpm. The process of preparation can be indicated as follows: (Scheme on the page 74).

Measurement of activity

The activity of all samples was measured by V. Oppltová of the Radiobiological Department of the Institute of Biology, Czechoslovak Academy of Sciences. She used mainly Friesecke and Hoepfner's apparatus; mica 1 mg./cm^2.or 0·99 mg./cm^2. The preparations were dried on stainless metal dishes or on aluminium foil. The solutions were evaporated and dried on them. Plants, both separated leaves and well-washed roots, were ground and the pulp was dried on aluminium foil. Insofar as any sap could be obtained by dripping from the pulp, this was dried on one foil and the remaining pulp on another.



In the case of leaves, in particular the dry weight of the older plants was greater; this caused variations in the thickness of the material; under these conditions the autoabsorption of the preparation was then strongly marked.

The background was calculated in every case from ten-minute measurements and gave 12 to 18, usually 14 cpm. Even measurements on different days varied to a relatively small extent. Otherwise the period of measurement was always 3 min.

Repetition of measurement of the same sample on different days showed close agreement (115 : 112).

On account of the relatively low activity of the samples, measurements were taken in different positions in order to be able to ascertain whether the number of impulses drops when the sample is at a greater distance.

Experiments with plants

Maize plants *(Zea mays)*, "horse tooth" variety, were germinated in Petri dishes in distilled water. They were then cultivated in Knop's solution and after five to eight days transferred to Knop's solution diluted by about one quarter. Radioactive humic acid with a total concentration of humate of about 0·01% was added to this solution. The activity of the preparations varied.

The plants were cultivated individually in test-tubes with 20 ml. of solution and the stand with the tubes was put into a large desiccator. Before the desiccator was opened the air was extracted through a safety flask, although the

experiments showed that under the experimental conditions no measureable amount of active carbon dioxide escapes.

At the start of the experiment the plants had roots of about 6 to 15 cm. (they were selected at approximately the same length in each series), coleoptiles were about 2 to 4 cm. long (sometimes the first leaf had broken through). During the experiment the plants grew normally, the daily increase for the roots being 1 to 2 cm., and side roots were also formed normally. The plants developed 2 to 3 leaves of normal green intensity during the experiment. Some plants formed adventitious roots over 10 cm. long at the base of the stem. The first two series were cultivated in the laboratory in diffused daylight. the rest in digestors under Tesla lamp TZ 20/R 36.

In the first series the solutions remained clear or were slightly clouded. In series V to VI the control solutions (without plants) remained clear, while the solutions in test-tubes with maize plants clouded within 2 to 4 days and there was a sediment of brown flakes. Control solution only clouded after over a month.

Examples of Experiments

II. Maize was cultivated in diluted Knop's solution with active humic acid preparation b. The activity of the residue from 20 ml. of solution at the start of the experiment (9. X. 1957), i. e. the amount for one plant, was 1320 cpm. Results of activity measurements are compared in the following survey:

Date	Days in solution	cpm./plant	
		roots	leaves
10. X.	1	4	0
14. X.	5	16	2
21. X.	12	75	14

Even after twelve days activity is weak, i. e. about 70 cpm/10 mg. for roots while for leaves it is about 5 cpm/10 mg. Adventitious roots of one plant formed after twelve days showed activity of 6 cpm, i. e. about 12 cpm/10 mg.

V.

Date	Days in solution	cpm. residue from 20 ml.	cpm./plant			
			Roots	Leaves		
				subst.	sap	total
21. XI.	0	3162	—			
23. XI.	2		41	8·6	5	14
26. XI.	5		128	19	15	34
29. XI.	8		232	7	5	12
3. XII.	12	4034	221	5·2	11·5	17

These values after eight to twelve day correspond for roots to about 300 cpm/10 mg., for leaves there are only single impulses.

VI. Humic acids prepared 1. XI. 1956 (preparation a).

Date	Days in solution	cpm. residue from 20 ml.	cpm./plant				
			Roots		Leaves		
			in solution	advent.	subst.	sap	total
17. XI.		2202					
19. XI.	2		58	—	3·7	0·3	4
22. XI.	5		132	—	2	3	5
29. XI.	12	2450	177	3	16	6	26

VII. Following up the experiments on resorption of humic acids from solution by roots we tried to observe the activity after the addition of humic acid through the leaves. We used two methods, the application of drops of the solution on the leaves, and according to Němec's method, by piercing the maize mesocotyl with a capillary filled with a drop of the solution and containing the suspension of humic acid.

The maize plants in this experiment had roots 15 to 25 cm. long, the first three leaves were developed; they were cultivated individually in test-tubes with 20 ml. of Knop's solution diluted to a quarter. From 22. XI. to 27. XI. the plants grew normally.

VII. a. A drop (0·01 to 0·1 ml.) of solution with a suspension of active preparation c was placed on the lower surface of the first leaf. It was difficult to keep the drops on the smooth leaf surface, but those which stayed dried on well. After five days the first leaves with these drops were dried for autoradiography, while the younger leaves and the roots were ground as before and dried on folio and measured; these upper leaves and the roots in solution did not come into direct contact with the active preparation and activity could spread to them only from the first leaves.

The average of 15 measurements gave a value of 3·7 cpm. in roots and 2·7 cpm in leaves, i. e. an activity within the bounds of measurement.

VII. b. 0·05 to 0·1 ml. of solution with a suspension of the active preparation was drawn into the capillary, which was thrust into the mesocotyl of maize or above the mesocotyl. The results of measurements taken after seven days were 6·2 cpm in roots and 9 cpm in leaves.

Autoradiograms

Autoradiograms fully confirmed the results obtained by measuring with the counter.

According to the intensity of radiation the roots were printed slightly grey to nearly black on the autoradiograms, while activity hardly spread at all to

the leaves so that they were not shown at all on the radiograms. It was only after 12 days that faint autoradiograms of the leaves began to show as hardly noticeable shadows after one month's exposure on the X-ray films Foma.

It is also necessary to deal with the question of the possibility of adsorption of active compounds at the surface of the roots. From autoradiograms it is often possible to observe increased activity at the surface. Localised structures within the root tissues, particularly meristems, young lateral roots, and vascular bundles show much more intensively black. Active compounds do not spread from the roots in a regular manner. When their time of action is short the part of the root above the solution is less clearly shown, the lower parts of leaves are not shown at all and only the tips of the leaves are clearly black.

In the leaves, both from cultures with active bicarbonate and from those with humic acids, the vascular bundles are the first and most intensively marked.

Since the humic acids are easily precipitated from solution, it is necessary to count with their adherence to the root surface. Autoradiograms show that sometimes, in isolated cases, places appear on the root surface which are strongly black, thus indicating localised precipitation.

Discussion

From the literature quoted and from our experiments it is evident that radioactive carbon in carbon dioxide or from a solution of inorganic salts (bicarbonates) penetrates very rapidly into plants. According to Kursanov (1957) radioactive carbon spreads in herbaceous plants at a rate of 3 cm. min., that is 2 m./hour. In recent literature there is a wealth of evidence on the rapid spreading of radioactive carbon in plants. In volumes of Plant Physiology for recent years, and elsewhere, very many different experiments with various isotopes have been described (cf. M. v. Ardene).

On the other hand, with maize plants cultivated in a solution of active humic acid the roots, even after five days, showed a relatively low activity and only after 12 days did it rise. From the total amount of radioactive carbon in the solution however, only a small part was resorbed. In the leaves, even after the end of this period, the activity was at the border-line of measurement or weak. Thus, active compounds spread from the roots into the leaves very slowly. This is also confirmed by the behaviour of adventitious roots, which grew above the level of the solution into the damp air and were either not radioactive or were radioactive to a much lesser extent than roots immersed in the solution.

The activity of sap pressed from the pulped leaves (unifiltered) varied greatly; however, it is not possible to say that activity accumulates in them: either a considerable part of the radioactive compounds in the leaves was not dissloved or they remained adsorbed on the solid residue of the leaves.

Kuzin and Merenova have studied plants, which had grown for 7 days on soil to which radioactive organic matter had been added (1955). In 1955 they already demonstrated that carbon is taken up by the roots from dissolved organic compounds and rapidly conveyed to the leaves, while on the contrary

plants sown on soil with insoluble components of green manure took up radioactive carbon slowly ("medlennoe usvoenie") (p. 251).

Our experiments do not, however, enable us to speak of permeability of cells to humic acid, i. e. if it penetrates unchanged into the cell. The only conclusion we can draw from them is that if we add radioactive humic acid to water or to nutrient solution radioactivity appears in the roots and later weakly in the leaves. If we assume that the activity measured in the plant organs was caused directly by the applied humic acid, it means that the acid penetrates slowly into the plant, that it does not accumulate and spreads from the roots slowly. It is necessary to consider the possibility that radioactive carbon gets into the plant in the humic acid molecule, but the possibility cannot be excluded that it gets into the roots and from them to the leaves only after the disintegration of large molecules, or even after their mineralization. We must consider the possibility that humic acids added to the solution may be decomposed by exoenzymes from the roots or by microbial activity and reach the roots only following this. But in this case, too, they get the plant slowly. In the meantime it is difficult to bring this fact into line with the repeatedly confirmed experience that the influence of humic acids on the growth of roots is exerted positively from the first hours or days.

Since the activity of the nutrient solution was increased during cultivation, the plant does not take up even that quantity of humic acids which was dissolved in the corresponding volume of transpired water; it can perhaps be judged from this that plants took up large molecules and that for this reason the process was slow. It is definitely not possible to speak of accumulation in the tissues.

This fact points to the possible conclusion that during cultivation humic acid was not mineralized as far as carbon dioxide, because this could escape into the air and would be taken up by the plant much more quickly. Alternatively, mineralization is so slow that the activity of its products is very low.

References

Ardene, M. v.: Tabellarische Darstellung zur Anwendung von Radioisotopen. — Berlin 1957.

Horáček, J. a Grunberger, D.: Rychlá modifikovaná metoda na stanovení ^{14}C. (A rapid modified method for the determination of ^{14}C.) — Chemické listy **51** (10) : 1944—1946, 1957.

Kursanov, A. L.: Ispolzovanie v SSSR radioaktivnikh izotopov v biologii i selskom khozyaystve. Doklady, predstavlenniye SSSR na mezhdunarodnuyu konferenciyu po mirnom ispolzovaniyu atomnoy energii. (The application of radioactive isotopes in the USSR in biology and farming. Papers presented by the USSR at the International Conference on the peaceful uses of atomic energy.) — Moskva 1955.

Kursanov, A. L.: Recent advances in plant physiology in the USSR. — Ann. Rev. Plant Physiol. 7 : 401—436, 1956.

Kursanov, A. L.: The root system as an organ of metabolism. Internat. Conference on Radioisotopes in Scientific Research. — Unesco (NS)RIS/**128** : 1—12, 1957.

Kuzin, A. M., Merenova, V. L.: Ob usvoenii rasteniyami ugleroda iz organicheskikh udobreny. (On the assimilation of carbon from organic manures by plants.) — Tr. Inst. biol. fiziki AN SSSR **1** : 247—255, 1955.

Liebster, J., Chrastil, J., Babický, A.: Příprava glukosy ^{14}C fotosyntesou. (The preparation of glucose ^{14}C by photosynthesis.) — Čs. biologie (Praha) **6** (2) : 150—154, 1957.

Prát, S., Čatský, J., Melichar, O.: Vliv humusových látek (oxyhumolitu) na rostliny. (The effect of humus substances (Oxyhumolits) on plants.) — Acta Soc. Bot. Pol. **26** (2) : 325 — 347, 1957.

Prát, S.: Versuche mit radioaktiver Huminsäure (C^{14}). Vortrag IV. Internat. Kongress f. Biochemie 1.—6. IX. 1958 Wien.

Prát, S.: Pokusy s huminovými kyselinami s ^{14}C. These referátů Symposia o účincích ionisujícího záření a použití radioisotopů v biologii a medicině (81). (Experiments with humic acid ^{14}C. Synopsis of reports made in the symposium on the effects of ionizing radiation and on the application of radioisotopes in biology and medicine.) — Praha 1958.

Additional references. When the manuscript being edited, the following contributions were published:

Simonart, P., Mayaudon, J.: Étude de la décomposition de la matiére organique dans le sol, au moyen de carbone oxydatif. I. Cinétique de l'oxydation en CO_2 de divers substrats radioactifs. — II. Décomposition du glucose radioactif dans le sol. A. Répartition de la radioactivité dans les fractions humiques du sol. B. Répartition de la radioactivité l'α-humus. — Plant and Soil **9** (4) : 367—384, 1958.

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Гуминовые кислоты с C^{14}

С. ПРАТ, Ф. ПОСПИШИЛ

Резюме

1. Листья сахарной свеклы (Beta saccharifera), которые ассимилировали активный углекислый газ $C^{14}O_2$, подвергались процессу гумификации в земле. После 3—5-месячной гумификации высушенная почва экстрагировалась смесью эфира со спиртом и потом гуминовые кислоты отделялись от фульвокислот с помощью едкого калия, или едкого натриа или соляной кислоты. Все фракции оказывались радиоактивными.

2. У растений кукурузы (Zea mays), выращенных в разведенном питательном растворе Кнопа с прибавлением активной гуминовой кислоты. в корнях и листьях наблюдалась радиоактивность. Однако между корнями и листьями была большая разница. Если активность корней через 2—12 дней составляла около 100—200 имп./мин., то активность листьев была около 20 имп./мин. в пересчете на 1 растение, или же около 300 имп./мин./10 мг у корней и только следы активности наблюдались у листьев.

3. Когда на нижнюю часть обратной стороны первого листа кукурузы наносили каплю раствора и суспензии активной гуминовой кислоты, активность весьма незначительно распространялась и в этом листе. Активность второго и третьего лисета через 5 дней бывала на пороге измераемости так же и в корнях. Из капилляров, воткнутых в мезокотиль, гуминовая кислота распространялась таким же образом и через 5 дней наблюдались лишь следы активности.

4. Авторадиограммы полностью соответствовали результатам, полученным с помощью счетчика. Корни вырисовывались отчетливо и более или менее интенсивно, тогда как листья не появлялись или появлялись только в виде слабых теней.

5. Так как активность питательного раствора в течение культивации повышалась, растения не впитали даже того количества гуминовой кислоты, которое было растворено в объеме воды, выделившейся при транспирации. Можно полагать, что в растения поступали крупные молекулы и поэтому скорость их поступления была медленной. Во всяком случае нельзя говорить о накоплении в тканях.

6. Наши опыты не дают ответа, проникает ли гуминовая кислота в клетки в неизменном виде. Можно только сказать, что, если радиоактивную гуминовую кислоту

прибавить к воде или к питательному раствору, то радиоактивность появляется в корнях и позднее и слабее — и в листьях. Даже если допустить, что отмеченная нами в органах растений активность была обусловлена прямо применением гуминовой кислоты, это означает, что гуминовая кислота проникает в растения медленно, не накапливается и из корней медленно распространяется по всему растению.

Возможно, что радиоактивный углерод попадает в растение в молекуле гуминовой кислоты, но не исключена возможность, что он попадает в корни и из корней в листья только после распада крупной молекулы, или же только после ее минерализации. Однако при быстрой минерализации активность, несомненно, распространялась бы в растении быстрее.

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